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# BIO-CHEMICAL DECOMPOSITION OF CELLULOSIC MATERIALS, WITH SPECIAL REFERENCE TO THE ACTION OF FUNGI<sup>1</sup>

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(With Plates I-IV, 7 Graphs and 1 Text-figure.)

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## I. INTRODUCTION.

THE problem of the decomposition of cellulosic materials has hitherto been attacked along two different lines. On the one hand there have been attempts to correlate the rate of decomposition with the chemical constituents of the materials. On the other hand, the discovery that micro-organisms are agents of such decomposition has led many investigators to direct their attention to the study of the behaviour of these organisms.

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Wollny<sup>(44)</sup> found that amounts of nitrogen contained in organic materials controlled the rate of decomposition. Rahn<sup>(27)</sup> showed that the application of nitrate or ammoniacal nitrogen accelerated the decomposition of straw. This point was further developed by Hutchinson and Richards<sup>(16)</sup> when they first revealed the quantitative combination of nitrogen and carbohydrates. According to these workers, the chief necessity for the most rapid breakdown of straw is a supply of assimilable nitrogen compounds in suitable concentration under aerobic and neutral or slightly alkaline conditions. They further found that nitrogen thus applied in a soluble condition is temporarily immobilised, and that the amount of nitrogen that straw is thus capable of locking up is equal to that necessary for pronounced rotting. The amount of soluble nitrogen temporarily immobilised by 100 parts of dry straw or other cellulosic material is termed the "nitrogen factor." Richards and Amoores<sup>(29)</sup> found that even in spite of the addition of assimilable nitrogen, certain materials such as sawdust, rice-husk, old bracken or coconut-shell cannot be decomposed, while some others such as banana stem or hop-bines take a long time for decomposition.

Dvorak<sup>(10)</sup> stated that materials rich in oxygen and poor in carbon decomposed more rapidly than those rich in carbon and poor in oxygen.

Starkey<sup>(35)</sup> while acknowledging that the rapidity of decomposition of some crude organic materials, such as rye and alfalfa, may be associated to some extent with their nitrogen content, does not consider nitrogen to be the limiting factor. Dextrose was decomposed most rapidly of all the materials studied, some rich in nitrogen; and cellulose decomposed the most slowly. Thus, apparently, no broad generalisations have been found to apply to the relative ease of decomposition on the basis of carbon, nitrogen and oxygen contents.

A detailed study on various constituents of straw was carried out by Hebert<sup>(13)</sup> who found that they decomposed in the following order: chlorophyllous matter, gums, tannins, glucose, dextrin, higher carbohydrates (cellulose and straw-gum) and finally vasculose. Van Suchtelen<sup>(39)</sup> states that in general the less abundant hexoses and pentosans decompose first followed by the polysaccharides, celluloses, pectins, starches and albumins. A strongly resistant carbonaceous residue is left, which however is decomposed very slowly. Dvorak<sup>(10)</sup> finds that fresh plant materials are the most available largely because the lower carbohydrates are more abundant; in the older plants, ligno-celluloses predominate. Richards and Hutchinson<sup>(28)</sup> in their patent, claim that all carbonaceous materials containing an adequate total quantity of carbo-

hydrates (30 per cent. and upwards) such as starch and pentosans and preferably not too high a proportion of ligno-cellulose are fermentable in the presence of assimilable nitrogen.

The study of micro-organisms active in the decomposition of organic materials dates from the time of Mitscherlich (23) who is generally considered to be the first to attribute the decomposition of organic materials to bacteria. Since that time bacteria have been given a great deal of prominence, and till the beginning of this century, they were considered the sole agents responsible for the disappearance of organic matter in the soil.

A great deal of work has recently been done on the activities of fungi in relation to soil fertility, and though their activities appear, perhaps, to be not so varied as those of the bacteria, fungi are important contributors to the ammonification, nitrogen transformation, cellulose decomposition and humification processes. A detailed survey of the literature pertaining to these problems has been given by Waksman (40).

The problem of the decomposition of cellulosic materials is thus bound up not only with the investigation of the chemical constituents of the organic materials, but also with the behaviour of the micro-organisms towards these constituents. While the resistant nature of the compounds of nitrogen present in these materials has been definitely proved by many workers, information on the availability of the carbonaceous compounds during this process is incomplete. The present investigation is therefore directed to the study of the decomposition of carbonaceous compounds in presence of assimilable nitrogen, and also to the study of different micro-organisms most active in such decompositions as regards their behaviour towards these constituents.

## II. OUTLINE OF THE SCHEME OF WORK.

The experimental work naturally falls into three parts:

### A. *A quantitative study of different carbonaceous compounds during decomposition.*

The main carbon constituents of plants may be classified as (1) celluloses, (2) hemi-celluloses<sup>1</sup>, (3) starches, (4) sugars, (5) pentosans, (6) lignins.

Two materials of different susceptibility to decomposition were selected for detailed study. One, rice-straw, is decomposed rapidly,

<sup>1</sup> No attempt is made to determine hemi-celluloses, as, though not of constant composition they mostly consist of pentosans, and these are separately estimated.

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while the other, poplar wood, is attacked with difficulty by micro-organisms and is a good example of a resistant material.

In the case of rice-straw, the material was allowed to rot and periodical short time analyses were carried out for the above mentioned constituents as well as for ammoniacal and protein nitrogen. In the case of poplar wood different carbon compounds, for example sugars, starches and pentoses, were added and their effect on decomposition was observed. Various other materials were examined for their different carbon constituents, especially pentosans, celluloses and lignin, in order to discover a possible correlation between these and the "decomposability" of the materials.

Other experiments were carried out under the same conditions with pure constituents. For this purpose pure cellulose, in the form of filter paper, was moistened with purified preparations of hexoses and pentoses. The experiment was intended to indicate which of these constituents might assist the decomposition of raw materials.

##### *B. A study of the relative importance of bacteria and fungi in decomposition.*

Bacteria and fungi were isolated from a decomposed manure heap and were separately inoculated into the rice-straw. The results of their activities were quantitatively determined and the products of decomposition were submitted to nitrification tests.

##### *C. A study of the life history and physiological behaviour of certain fungi which were found (vide section B) to play the more important part in the process.*

Three fungi very active in these processes were isolated and their morphology and physiology were studied especially in relation to plant constituents.

#### III. METHODS OF ANALYSIS.

The following methods of analysis were adopted throughout the experimental work.

*Furfuroids.* The standard method, Krober and Tollens<sup>(1)</sup>, for the determination of pentoses and pentosans was employed. The furfural obtained by distillation was estimated as phloroglucide. All figures are expressed as pentosans.

*Dextrose* as well as *Invert Sugars* were estimated in the alcoholic extracts by the iodimetric method<sup>(1)</sup>.

*Starch* was at first determined by the hydrolysis method (1). Two gm. of the material, well washed to free it from reducing sugars, were heated on the water bath from two to five hours with 200 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1.125). A portion of the filtrate was distilled with hydrochloric acid for pentosans, while in the other portion, the copper reducing power was determined by the iodimetric method. The copper reducing power of starch was obtained by subtracting the equivalent of pentosans from the total, but compared with the Taka-diastase method (9) the figure thus obtained was higher—perhaps as a result of the hydrolysis of some other constituent. In the case of fermented materials both these methods gave quite untrustworthy results.

*Water-soluble and alkali-soluble portions* (11). Two gm. of the material were heated on the water bath for one hour with 100 c.c. of distilled water, filtered, washed with hot water, dried and weighed. The loss in weight gives the figure for water soluble substances. The residue was treated on the water bath for half an hour with 1 per cent. alkali (treatment as in the chlorination method for cellulose estimation), filtered, washed with dilute acetic acid and then with hot water till free from acid, dried and weighed. The difference between this weight and that of the water-soluble portion represents alkali-soluble substances.

*Cellulose* was estimated by the chlorination method of Cross and Bevan (8). Two gm. of the roughly ground material were heated on the water bath for 20 minutes with 100 c.c. of 1 per cent. NaOH solution, the volume throughout being kept constant by the addition of water. Owing to the difficulty in filtration, especially with decomposed materials, the Gooch crucible in Sieber and Walter's (33) apparatus was replaced by a small Buchner funnel. Three chlorinations of 15, 7 and 5 minutes were found sufficient for the purification of cellulose. After each chlorination a little sulphurous acid was added to stop any further action of chlorine, the excess being removed by filtration and washing. The contents were then washed into a beaker with a jet of hot water, made up to known volume and heated on the water bath for 30 minutes with 3 per cent. sodium sulphite solution. After the third chlorination and treatment with sodium sulphite, it was filtered through a Gooch crucible and thoroughly washed. The contents were then flooded with 2 per cent. permanganate solution for five minutes, filtered, washed, treated with sulphurous acid and finally washed until free from acid. The residue from undecomposed materials was white. In the fermented materials, the first chlorination turned it darker, the colour being reduced with further chlorinations. But in the end it retained a greyish

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colour which was constant in spite of either additional chlorinations or treatment with permanganate and sulphurous acid. Its freedom from lignin could be verified by treatment with 42 per cent. hydrochloric acid.

It was found inadvisable to have the material finely ground for chlorinations. Besides causing difficulties in filtration, the particles, owing to their fine nature, came in very close contact with each other, thus making the free passage of chlorine very difficult. In such cases even three chlorinations were found insufficient for the removal of impurities.

All the figures are calculated on the cellulose free from ash.

*Lignin.* Attempts were made at first to get the figure for lignin by difference. After the alkali treatment in the chlorination method, the residue was dried in the steam oven and weighed. The difference between this weight and that obtained after chlorination was considered to represent lignin. But as this was found to give very low figures it was replaced by Willstatter and Zechmeister's method (41). The figures are calculated on lignin free from ash.

*Total Nitrogen* (1) was determined by the Kjeldahl method, while for ammoniacal nitrogen (1), the material was distilled with magnesium oxide.

### IV. EXPERIMENTAL.

#### A. *A quantitative study of different carbonaceous compounds during decomposition.*

##### Series I. Decomposition of rice-straw.

A preliminary analysis of rice-straw gave the following results:

Dry matter	100	gm.	Invert sugar	0.6	gm.
Organic matter	82.67	„	Pentosans	24.6	„
Cellulose	45.46	„	Lignin	10.06	„
Starch	0.7	„	Total nitrogen	0.41	„
Dextrose	0.65	„			

A number of preliminary experiments in decomposition were carried out to ascertain the best temperature, the best interval between two analyses and the most important constituents requiring special attention.

A comparative study at two temperatures of 35° and 50° C. showed the former to be suitable for investigation, the decomposition being slow enough to enable the disappearance of various constituents to be traced. Further, it was also found that an interval of about four days between analyses was essential to give an idea of the relative importance of various compounds.

During the course of these experiments, it was found that the determination of sugars did not give a fair index of their disappearance during the course of decomposition due to the formation of these sugars as intermediate compounds. To measure their importance, the alternative method of elimination had therefore to be employed. It was thus found that the removal of sugars by cold water lixiviation, which extracted 93 per cent. of the total quantity, had no effect on the rate of decomposition.

As regards starch, its presence in the straw in minute quantities, the difficulty of its estimation in the rotting materials due to the lack of any reliable method, as well as the impossibility of using the method of elimination which in this case necessitates autoclaving with consequent structural changes, force us at present to give up the idea of tracing its decomposition. The resistant nature of lignin shown by various workers as Mahood and Cable<sup>(20)</sup> and Johnsen and Hovey<sup>(17)</sup> proves its non-importance as microbial food. Thus the only constituents which require careful consideration are pentosans and cellulose and in this series their decomposition is studied under the best conditions of temperature and period of analyses. The procedure was as follows.

Twenty gm. of chaffed rice-straw, obtained from the Punjab, were accurately weighed, slightly moistened with water by a sprayer and allowed to remain in this condition for about 10 minutes. Ammonium carbonate solution, equivalent to about 1 gm. of nitrogen<sup>(16)</sup> to 100 gm. of straw was added drop by drop from a burette and thoroughly mixed with the straw. After the addition of inoculum from old decomposed manure the straw was bottled and enough water was sprayed to cause thorough wetting. The bottles were incubated at 35° C. and analyses were carried out every four days. The portions for ammoniacal nitrogen, total nitrogen and dry weight were taken immediately. The rest of the material was thoroughly dried in the steam oven and kept for other determinations.

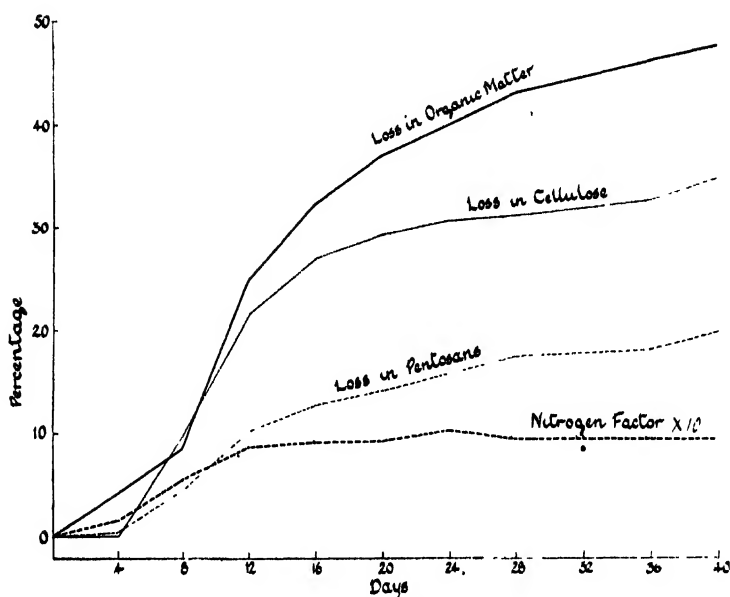
The fungi were seen on or about the 7th day. They continued their vigorous growth till the 20th day when they began to sporulate. By the 24th day sporulation was complete, and by the 28th day, no trace of fungus hyphae could be seen by the naked eye. Microscopic examination on the 36th day showed no hyphae, though spores were observed in plenty. No water-logging was observed except in one case.

The results are given in Table I and also represented graphically.

*Discussion.* It is quite evident from the results, that during the first fortnight there is a rapid loss of dry matter as well as assimilation of



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Graph 1. Decomposition of Rice-Straw at 35° C.

Table I.

### *Decomposition of rice-straw at 35° C.*

Figures calculated on 100 gm. of original dry matter.

	At start	4 days	8 days	12 days	16 days	20 days	24 days	28 days	32 days*	36 days	40 days
Dry matter	100	95.57	91.2	75.4	69.8	63.9	60.8	58.6	52.9	56.8	55.0
Organic matter	82.67	78.37	74.1	57.8	50.85	45.8	43.08	40.05	33.9	37.0	35.5
Total nitrogen	{0.41 1.13}	1.3	1.38	1.37	1.35	1.35	1.45	1.36	1.31	1.36	1.33
Ammonia nitrogen	1.13†	0.78	0.44	0.086	0.037	0.024	0.014	0.019	0.059	0.021	0.023
Nitrogen factor†	—	0.12	0.53	0.88	0.90	0.92	1.02	0.93	0.84	0.91	0.90
Pentosans	24.78	24.65	20.6	14.33	12.5	10.65	9.08	7.42	Not determined	6.8	5.32
Fraction soluble in water	14.1	14.95	12.8	16.4	15.1	13.9	13.2	13.25	„	12.3	11.7
Fraction soluble in alkali	32.2	26.85	34.9	27.3	26.9	23.6	23.0	20.55	„	20.5	20.3
Residue	53.7	53.7	43.5	31.7	27.8	26.4	24.6	24.8	„	24.0	23.0
Cellulose	45.46	45.88	35.8	24.05	18.7	16.75	15.17	14.8	„	13.1	11.1
Fungus growth	—	Nil	Just appearing	Vigorous	Vigorous	Vigorous	Sporulating	Spores	Not visible	Not visible	Not visible

\* Water-logged. H<sub>2</sub>S small.

† Added as (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>.

‡ This is the amount of available nitrogen temporarily immobilised by 100 gm. of straw (16).

ammonia. The visual inspection of bottles showed the presence of fungus hyphae on the 6th or 7th day and this is immediately followed by the rapid loss of dry matter as the figure for the 12th day indicates. Table I and Graph I clearly indicate that so long as the fungi were vigorously growing, disappearance of dry matter and assimilation of ammonia were at their highest. More than 60 per cent. of the dry matter was lost and nearly 90 per cent. of the ammonia was assimilated during that period.

Further, the loss of pentosans shows a striking correlation with the fungus growth. (The physiological studies of fungi show that cellulose is a poor nutrient food for them.) During the first four days, there is no loss of pentosans at all, but between the 4th and 8th day, when the fungus growth must have started, the loss of dry matter is represented almost wholly by that of pentosans (see Graph 1). It seems, from the results after the 12th day, that the fungi have a tendency to store these pentosans. *Coprinus* sp. separated from the decomposing straw gave 7.82 per cent. pentosans on dry weight. During the period of vigorous destruction of straw this fungus alone is found to form an appreciable part of the whole material. In one case 151 gm. of the wet material gave 17 gm. of this fungus or nearly 11 per cent. Thus pentosans seem to be a very suitable food for fungus growth.

It must not be argued from this that pentosans are the chief food materials for fungi. Later work shows that they grow equally, and in some cases even better, on some sugars and starches, but as a rule, waste plant materials contain very little of these; indeed rice-straw is very deficient in both. It can therefore be safely concluded that fungi are playing an important part in this process.

As regards losses of various constituents, more than 80 per cent. of the pentosans disappeared during the 40 days of the experiment. The loss of pentosans, especially during the early stages of decomposition, is striking, and suggests, as discussed above, the importance of these compounds for the activities of micro-organisms. Cellulose has also suffered a great loss. Apparently the loss in cellulose is even greater than in pentosans. But the study of Table II shows that real loss is less as the rapid loss of cellulose is followed by an increase in the alkali and water-soluble portions. It is, therefore, quite likely that cellulose passes through these stages of decomposition.

Lisse<sup>(19)</sup> in his study of rotting wood found the increase to occur in the alkali-soluble portion which according to him indicates the formation of hemi-cellulose. Bray and Staidal<sup>(3)</sup>, in their study of the chemical

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Table II.

*Showing the stages through which cellulose passes during decomposition.*

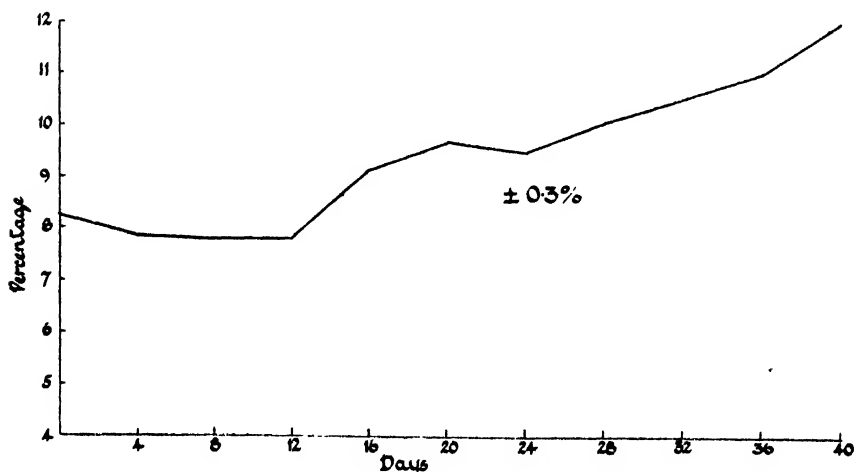
Calculated on 100 gm. of original dry matter.

Days	Loss in cellulose	Loss or gain in water and alkali-soluble portions	Total loss or gain	Loss in organic matter
4	0	-4.5	-4.5	-4.3
8	-10	+6.0	-4.0	-4.27
12	-11.9	-4.0	-15.9	-16.3

N.B. Since at later stages of decomposition the cellulose itself suffered loss during analysis, the figures for these values after the 12th day are not given.

changes involved during infection and decay of wood and wood-pulp, found an increase in cold and hot water-soluble portions as well as in alkali-soluble associated with a decrease in the amount of cellulose. Various authors who have devoted their attention to the decay of wood-pulp find that the highly complex celluloses, *e.g.*  $\alpha$ -cellulose, pass into others of increasing simplicity as  $\beta$ - or  $\gamma$ -cellulose or hemi-celluloses, till at last all pass into  $\text{CO}_2$ , water and perhaps hydrogen and methane.

Further, the gradual increase observable after the 16th day in the difference between the residue after alkali treatment and the cellulose



Graph 2. Percentage difference between residue after alkali treatment and cellulose after chlorination, showing that at later stages of decomposition cellulose is attacked by chlorine.

figure obtained after chlorination proves that cellulose becomes less resistant to this chlorination treatment during decomposition. As this difference mostly represents lignin, the other constituents being almost

removed by alkali treatment, it should never show such an increase. On the other hand, accompanying the breakdown process, there should be a decrease as the humus formed from lignin<sup>(12)</sup> is easily removable by treatment with alkali. As indicated in the description of the method of chlorination, the material gets darker with the first chlorination, and though the colour is reduced with successive chlorinations, the residue is never white as in the case of original straw. It seems, therefore, that the cellulose itself becomes weaker and more easily susceptible to the attack of chlorine. Consequently the figure obtained represents less cellulose than is actually present in the material and it is very difficult to get an idea of the real loss of this constituent. None the less, it definitely shows that the greater portion of cellulose is not resistant at all and is even susceptible to the attack of micro-organisms during the first stages of decomposition.

#### Series II. Decomposition of poplar wood.

In this series attempts were made to decompose woody material. Poplar wood was chosen for experiments. These experiments confirmed the general experience that the woods are not easily attacked by micro-organisms even in the presence of available nitrogen.

A preliminary analysis of poplar wood for important constituents<sup>1</sup> gave the following results:

Dry matter	100.0 gm.	Pentosans	20.63 gm.
Organic matter	98.32 ..	Lignin	28.44 ..
Cellulose	66.3 ..	Total nitrogen	0.31 ..

As is evident from the above table, there is a certain overlapping in the figures for the different constituents, *e.g.* in the case of pentosans and cellulose. The same may be expected in the case of rice-straw.

The experimental technique for the decomposition of this material was exactly the same as before. 20 gm. of the wood-shavings were used for each experiment and available nitrogen was added in the form of ammonium carbonate. As woods are rather deficient in ash, a definite quantity of mineral salt solution<sup>1</sup> was added. A control set was incubated at the same time. To both, 1 gm. of calcium carbonate was added.

The bottles with mineral salts showed the fungus growth on about the 6th day, while those without showed only a slight growth after a

<sup>1</sup> Composition of mineral salt solution:

NaNO <sub>3</sub>	2.5 gm.	CaCl <sub>2</sub>	0.1 gm.
K <sub>2</sub> HPO <sub>4</sub>	1.0 ..	FeCl <sub>3</sub>	0.02 ..
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.3 ..	Distilled water	1000 c.c.
NaCl	0.1		

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fortnight. In none of the bottles was the action as vigorous as with rice-straw. The experiment was stopped after two months and the contents were analysed as in the previous series.

Table III.

*Decomposition of poplar wood at 35° C., for two months.*

Figures calculated on 100 gm. of original dry matter.

	Wood gm.	Wood + mineral salts gm.
Loss of organic matter	19.77	37.14
Ammonia nitrogen	0.11	0.036
Total nitrogen	0.68	0.96
Nitrogen factor	0.26	0.62
Loss of pentosans	—	46.1
Loss of cellulose	—	63.2

Similar experiments, made with pine wood shavings, gave no evidence of decomposition even after two months: after this period the loss of dry matter was found to be about 3 per cent. No further analysis was therefore made.

*Discussion.* These results clearly show that lack of minerals is one of the essential factors inhibiting decomposition. The addition of minerals alone has almost doubled the loss of dry matter and more than doubled the nitrogen factor. This again lends support to the theory that fungi play an important part in the decomposition. The controls showed very poor growth of fungus flora and the loss of dry matter as well as the nitrogen factor are quite insignificant.

As regards loss in various constituents, that in cellulose should be accepted with qualification for the reasons already stated. But even after making allowances for these objections, cellulose has suffered a great loss, even greater than pentosans. The small loss in pentosans is rather surprising. According to the previous experiment with rice-straw, pentosans suffer the greatest loss during the early stages of the process; but with poplar wood, this does not appear to be the case, for even after two months' fermentation only 50 per cent. of the pentosans has disappeared. Two possible explanations may be advanced.

(1) *The difficulty experienced by organisms in getting access to the food materials.* It is a fact well established, that the cell-walls of wood and straw are lignified, the amount of lignification varying greatly in different groups of higher plants. For instance, woods are far more lignified than straws. The process of lignification, according to Sachs (31), consists in a series of progressive and intrinsic modifications of a cellulose or oxy-

cellulose tissue, the final products of metabolism (aromatic products, pentosans, etc.) being split off from the fundamental tissue substances and excreted. The distribution of these non-cellulosic constituents is at present a controversial point. Wislicenus(43) has tried to explain it by the adsorption theory, while there is another school of thought which, both by the study of the reactive groupings of the constituents of the ligno-cellulose(21) as well as the resistance offered by these non-cellulosic products to chemical treatments(32), considers these compounds to be chemically combined with cellulose. It suffices our purpose to know that the products of metabolism of the cell-wall remain intimately associated with the residues of the fundamental tissue. It is thus quite reasonable to conclude that micro-organisms have to attack this fundamental tissue to get their nourishment. Thaysen and Bunker(36), who have made a microscopic study of the destruction of cellulose fibres and fabrics by micro-organisms, come to the conclusion that destruction is from outside inwards. A microscopic study of sections of wood, inoculated with soil suspension, shows that organisms pierce the tissue at the point at which they happen to be present, and their appearance in the cell-wall itself supports the assumption that they do not obtain their food from a distance by enzymic activity.

Of the fundamental tissue, lignin is the most resistant, and therefore the rate of decomposition should be dependent upon the quantity of lignin present. The slow rate of decomposition of poplar wood and other woody materials may therefore be attributed to their high lignin content. Due to this resistant barrier, micro-organisms in woody tissues cannot easily obtain their food materials--pentosans. In the course of their activities, they attack the least resistant of the fundamental tissue components, viz. cellulose. This is probably the reason of the great loss of cellulose found during decomposition of poplar wood. But the slow activity of these organisms indicates that cellulose is not as good a food as some lower carbohydrates. The increased decomposition of plant tissues when in a state of fine division lends support to the theory.

To the above theory, however, there is one objection which is of a practical nature. In actual decomposition it appears that the rate of breakdown is not solely dependent upon the lignin content. This can be seen in the case of pine and poplar woods. The lignin content of poplar and pine respectively is 28.44 and 26.65 per cent. But while poplar comes under the category of slow decomposing materials, pine is hardly attacked at all. Thus there appears to be an additional inhibitive factor in pine wood. Whether this may be the lack of some necessary

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food material is discussed below. The possibility that the resinous compounds of pine may prevent the action of organisms has not been overlooked, but the works of Soderbaum and Barthel(34) and also of Migula(22) suggest that this may not be of importance.

(2) *Nature of furfural-yielding constituents.* In all previous experiments, the term "pentosans" is considered to be identical with furfural-yielding compounds. But analytical conversion into furfural, while specially characteristic of the pentoses and pentosans, is also a property of certain higher compounds such as glycuronic acid and probably oxycelluloses. It is claimed(8) that these types of cellulose, which are much more widely distributed in the plant world than the cotton type, yield furfural as a product of hydrolysis by hydrochloric acid. For example, 8.9 gm. of pentosans were obtained from the cellulose of 100 gm. of rice-straw, *i.e.* 33 per cent. of the total pentosans in this case is represented by some portion of the cellulose.

Though this differentiation may not be important chemically, it is likely to have a great physiological significance. This can be best illustrated by certain fermentation tests on the furfuroids. Cross, Bevan and Smith(7) in their researches on the carbohydrates of cereal straws have obtained the following interesting results.

Barley straw from Rothamsted plots was digested with 2 per cent. sulphuric acid under pressure and yielded an extract containing 70-80 per cent. of the total furfuroids in the straw. This was neutralised and fermented with yeast.

Furfuraldehyde (before fermentation)	...	.	38.0
„ (after fermentation)	.	...	4.7

Similar fermentation of the furfuroids obtained by hydrolysis of the straw cellulose with the same strength of acid at three and a half atmospheres pressure for 15 minutes gave the following results:

Furfuraldehyde (before fermentation)	...	...	39.7
„ (after fermentation)	...	...	26.1

Thus the hydrolysis product obtained in the two cases, though chemically similar, is not fermentable at the same rate. We have no definite idea as to the nature of the original material which has suffered this hydrolysis. So far, no method isolates it, in its original state, from cellulose. It is not, therefore, possible to get any direct evidence of its resistance to microbial attack. But we can come indirectly to certain conclusions by studying how these furfural-yielding bodies behave during decomposition.

The figures in Table IV were obtained as a result of the experiment in Series I and II. In the case of rice-straw in Series I estimation of pentosans in cellulose was done only on the 8th and 12th day as those days showed the greatest loss in cellulose.

Table IV.

*Loss of pentosans and cellulose at various stages of decomposition.*

				Loss of cellulose %	Loss of pentosans in cellulose %
Days					
Rice-straw (Series I)	.	...	8	21.3	9.0
"	"	..	12	45.0	40.0
"	"	...	40	75.6	73.0
Poplar wood (Series II)	...	...	60	63.2	52.4
Poplar wood rice-straw (Series III)			60	73.2	72.8

Table IV indicates that cellulose is even less resistant than the furfural-yielding bodies. Bray and Andrews<sup>(1)</sup> in their study of chemical changes of ground wood during decay also found that the loss in cellulose was greater than the loss in total pentosans. After three years' decomposition the figure for cellulose dropped down from 60 to 6.05 per cent. while that for pentosans was reduced from 12 to 2.56 per cent.

The chemical behaviour of these furfural-yielding bodies also shows their resistibility. Heuser and Haug<sup>(14)</sup> found that treatment with boiling 6 per cent. caustic soda did not reduce the xylan content of straw below 9.7 per cent. According to Winterstein<sup>(42)</sup> boiling with 5 per cent. sulphuric acid for three hours did not remove all the xylan. Although Heuser and Boedeker<sup>(15)</sup> have claimed to free the cellulose from pentosans by repeated extraction with hot 6 per cent. or cold 17 per cent. caustic soda, this drastic treatment led to the destruction of a considerable part of cellulose. Thus all the evidence, direct as well as indirect, points to the conclusion that these furfural-yielding bodies are more resistant than the true pentosans and perhaps some celluloses. As shown before, micro-organisms thrive well on available pentosans, and increase in resistant furfural-yielding bodies, therefore, would not be conducive to rapid decomposition. This is actually found in the case of poplar wood. Though the wood shows a large amount of total pentosans, about 50 per cent. of these belong to the category of resistant bodies. It would seem probable that this is the reason why even after two months such a large amount of pentosans is found in the decomposing material. In the rice-straw, on the other hand, less than 34 per cent. of the furfuroids are in this resistant form, so that more than



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66 per cent. may be regarded as easily available. It would be, therefore, reasonable to conclude that rice-straw would be more rapidly destroyed than poplar wood and actual tests support this view.

Extending the above theory further, we can postulate that the food factor is the limiting factor in the decomposition of the material, or in other words susceptibility to destruction is proportional to the amount of available food in any material. But though this is true in many cases, it is not applicable to all cellulosic materials. Some of these, containing equal amounts of assimilable pentosans, show great divergence in their susceptibility to decomposition, *e.g.* rice-straw and maize-straw. Both contain equal amounts of pentosans, but while the former is easily destroyed the latter is decomposed less easily. Thus we see that the food factor, like the inhibitive factor, though important, is not the only condition controlling decompositions of this nature.

It is quite clear from the above discussion that these two factors — inhibitive and food — are not independent. Both must be acting simultaneously and the predominance of the one above the other would determine rapid breakdown or otherwise of the material. In other words, if pentosans are much in excess of lignin, the material will be easily

Table V.

*Analysis of plant materials showing the relation of the ratio of pentosans and lignin to their "decomposability."*

Figures expressed on 100 gm. of dry matter.

	Total furfuroids expressed as pento- sans*	Cellulose by chlor- ination method	Furfuroids in cellu- lose as pentosans	Available furfuroids as pento- sans*	Lignin	Ratio of available pentosans to lignin	Decom- posability
Rice-straw	24.78	45.88	8.9	15.88	10.31	1.54	Rapid
Oat-straw	28.8	51.7	12.8	16.0	14.18	1.13	"
Barley-straw	28.4	49.4	11.2	17.2	16.16	1.06	"
Wheat-straw	31.28	52.1	15.15	15.13	14.57	1.04	"
Rushes	27.93	51.3	12.7	15.23	17.71	0.86	Slow
Maize-straw	25.83	45.0	10.6	15.23	20.34	0.75	"
White ash	22.33	53.4	9.26	12.07	28.38	0.43	Very slow
Poplar	20.63	66.3	10.95	9.68	28.44	0.34	"
Pine	8.97	57.41	5.9	3.07	26.65	0.12	Nil

\* Although "available" pentosans calculated as above are on the whole in the same order as total furfuroids, it is found that the actual order of "decomposability" runs more closely parallel with the "available" pentosans than the total furfuroids. A case in point is that of barley-straw. In this case while the ratio in the table puts it in the category of "rapid" which is also confirmed by actual tests, the ratio of total furfuroids to lignin would include it among the slowly decomposed materials.

decomposed, the rate of its decomposition decreasing as the ratio of pentosans to lignin decreases. If this be true, it should be easy to discover an index of the "decomposability" of a material by ascertaining the relative amounts of available pentosans (*i.e.* furfural-yielding bodies obtained by deducting furfuroids of cellulose from total furfuroids) and lignin. In Table V some of the materials are tested to ascertain this ratio as well as their "decomposability."

The results indicate that this test, while not giving exact figures, is quite satisfactory. It is possible to judge that any material having a ratio higher than 1.0 can be easily decomposed by micro-organisms; if the ratio falls between 1.0 and 0.5, the material is rather slow and so on. It is thus quite definite that both the factors—food and inhibitory—are inter-related, and that the presence of both, in certain definite ratios to each other, controls the rate of decomposition of plant materials.

Series III. Effect of carbohydrates on the decomposition of wood.

The previous experiments seem to prove that for a material to be easily decomposed the ratio of pentosan to lignin must be higher than 1.0. In this series attempts were, therefore, made to increase this ratio in poplar wood by the addition of various carbohydrates. The experimental technique was the same as before. Twenty gm. of the wood shavings were used for each bottle and each received 10 c.c. of mineral salt solution and 1 gm. of calcium carbonate. Straw gum was isolated by Tollens and Wheeler's<sup>(38)</sup> method. The arrangement of the series was as follows:

(1) Dextrose	...	...	...	...	...	1 gm. per bottle.
(2) Starch	...	...	...	...	...	1 "
(3) Xylose and arabinose in equal proportions	...	...	...	...	...	1 "
(4) Straw gum	...	...	...	...	...	1 "
(5) Rice-straw	...	...	...	...	...	5 "

Table VI.

*Decomposition of poplar wood at 35° C. for two months.*

Figures calculated on 100 gm. of original dry matter.

	Dextrose	Starch	Xylose arabinose	Wood gum	Rice- straw	Minerals only
Loss of organic matter	35.34	27.19	31.1	33.3	54.5	37.14
Ammonia nitrogen	0.08	0.09	0.08	0.096	0.007	0.036
Total nitrogen	0.83	0.73	0.79	1.12	1.004	0.96
Nitrogen factor	0.45	0.33	0.41	0.71	0.69	0.62
Loss of pentosans	—	—	—	—	72.6	46.1
Loss of cellulose	—	—	—	—	73.2	63.2

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All the cultures showed the fungus growth, but there was no visible decomposition except in the case of the rice-straw, which also retained moisture more efficiently than the others. The experiment was stopped at the end of two months and the materials were analysed (Table VI).

*Discussion.* The results are rather disappointing. The addition of food materials outside the tissue lowers the actual decomposition of the tissues. The loss of organic matter in all except rice-straw is less than that with minerals only. It seems therefore that the micro-organisms which obtained both their nitrogenous and carbonaceous food outside the tissues did not attack that lying within till this easily available supply was finished.

This is shown by the great loss that took place with rice-straw. In this case it was essential for the organisms to attack the tissues, and though they might have first attacked the straw, they afterwards attacked straw and wood equally. In fact, the material after two months was found to be a mass in which straw and wood were closely intertwined together by fungus hyphae. Even after deducting the figure for the loss of organic matter in which straw may be taken as 55 per cent. within two months, the wood alone shows a loss of 53.25 per cent. in this experiment. Thus though carbohydrates form good microbial food, their addition to plant materials does not in any way help decomposition of the latter.

### Series IV. Importance of hemi-cellulose in the decomposition.

We have left one important group of plant constituents called hemi-celluloses out of account because their composition is a matter of great controversy. It is so far known that these substances closely resemble the true celluloses, but are easily resolved into simpler carbohydrates by the hydrolytic action of enzymes and of dilute acids and alkalis. The carbohydrates thus obtained are xylose, arabinose, mannose and galactose. In our studies on decomposition we have given careful attention to the first two and as a result have come to the conclusion that they are important as microbial food. But our neglect of the other two would be a possible source of criticism against our theory, as they form in many plants an appreciable part of the hemi-celluloses. In fact, in the absence of any proof to the contrary it might be rightly claimed that hemi-celluloses and not pentosans alone may be the determining factor in the decomposition processes. This series is an attempt to test the validity of such criticism.

The difficulties in tracing the decomposition of hemi-celluloses are

obvious. Not only are these compounds complex materials of uncertain definition, but they differ in composition greatly in different plants. Thus the study of their decomposition in any one material would not lead us any further. It was therefore thought best to study the behaviour of their purified hydrolytic products. Thus xylose, arabinose, galactose and mannose were obtained in pure form and their effect on the decomposition of cellulose (filter-paper) was tested. The method of procedure was as in the last series. 15 gm. of chopped Whatman's filter-paper No. 1 were used for each bottle. These were moistened with 10 c.c. of mineral salt solution. Nitrogen was added in the form of ammonium carbonate. Carbohydrates were added in two doses to prevent high concentration. The plan of the experiment was as follows:

- |     |                        |                             |
|-----|------------------------|-----------------------------|
| (1) | Control (filter-paper) |                             |
| (2) | „                      | Mannose 0.75 gm. per bottle |
| (3) | „                      | Galactose „                 |
| (4) | „                      | Xylose „                    |
| (5) | „                      | Arabinose „                 |

All the bottles received 1 gm. of calcium carbonate. They were inoculated with a suspension from decomposing manure and incubated at 35° C. All except the control bottle showed fungus growth after about the 12th day. The control showed slight fungus growth after a month, but the decomposition in this was more rapid than in the other bottles. It continued to be vigorous all the time and at the end of the period (six months) only a small quantity of the material remained in the bottle. The decomposition in other materials was very slow. The series was stopped after six months and analysed. Except in the case of arabinose, all, including the control, had retained the original colour of the filter-paper. Arabinose showed browning of the residual material. The results are given in Table VII.

Table VII.

*Decomposition of cellulose in presence of carbohydrates at 35° C.,  
for six months.*

	Figures calculated on 100 gm. of original dry matter.				
	Control	Mannose	Galactose	Xylose	Arabinose
Loss of organic matter	82.1	19.15	22.2	39.8	48.8
Ammonia nitrogen	—	0.012	0.043	0.011	0.012
Total nitrogen	0.67	0.29	0.31	0.54	0.58
Nitrogen factor	0.63	0.24	0.23	0.49	0.53

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*Discussion.* (1) Mannose and galactose do not seem to be good microbial foods for the cellulose decomposing organisms under these conditions. The loss in organic matter and the nitrogen factor in both cases are very low. The low nitrogen factor, especially, indicates that the micro-organisms have not shown much activity in growth. Thus these compounds do not appear important in such decomposition under these conditions and their omission from our consideration would seem to be justified.

(2) This series supports the conclusion drawn from the previous one as regards the low rate of "decomposability" of the material when both carbon and nitrogen compounds are added. The micro-organisms do not attack the tissue in the presence of other more available food material.

### *B. A study of the importance of different groups of micro-organisms concerned in the process.*

In this section, attempts are made to investigate the relative importance of bacteria and fungi in the decomposition of plant materials. For this purpose sterilised straw was inoculated with pure cultures and the products of the activities of the several groups of micro-organisms were quantitatively estimated.

#### Series V. Decomposition of rice-straw by soil flora.

This series was devoted to the study of the combined activities of the soil population in the decomposition of rice-straw. It has been described in detail in Series I.

#### Series VI. Decomposition of rice-straw by fungi.

In this series, fungi in pure culture were inoculated into sterile straw and the products of their activities were analysed at periodic intervals as in previous series.

Twenty gm. of rice-straw were autoclaved in bottles under 15 lbs. pressure for half an hour on four consecutive days. In spite of the danger of changes in the straw, such autoclaving was found essential for complete sterilisation, steaming being insufficient to kill all the bacteria. Ammonium carbonate solution, in slight excess to what was theoretically required (1 part of nitrogen to 100 parts of dry matter), was diluted in separate flasks with water, just sufficient to wet the straw thoroughly and sterilised at 15 lbs. pressure for half an hour. It was found by a preliminary experiment that very little ammonia is volatilised in this way. These flasks were inoculated with the cultures

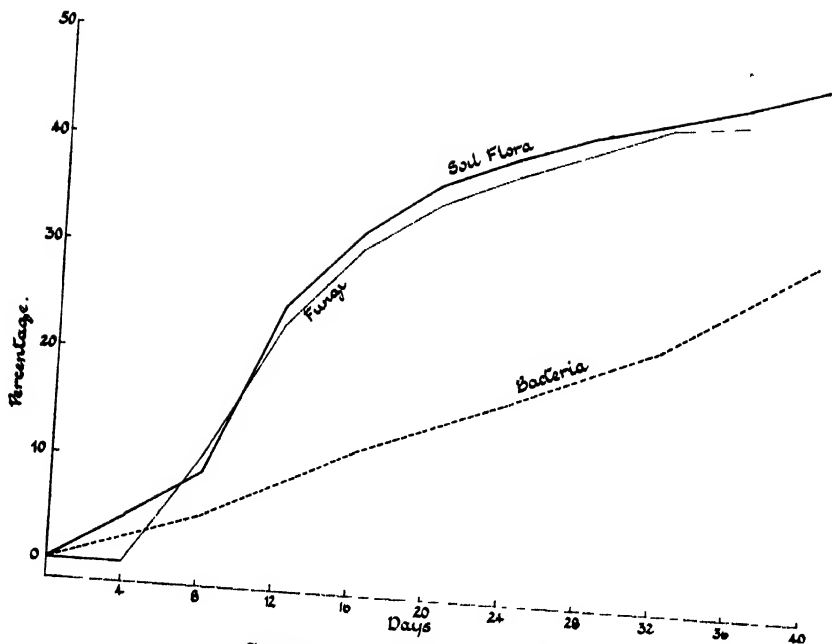
of three fungi (described in part C) grown on potato agar at 35° C.; they were then shaken for a few minutes and the contents were poured separately into each bottle. The inoculum consisted of spores in the case of *Aspergillus* sp. and *Acremonia* sp. and mycelium in the case of *Coprinus* sp. The bottles were incubated at 35° C. and analyses were carried out every four days. At the time of each analysis care was taken to test the material for bacterial infection, both microscopically and by plating on Thornton's medium (37).

Fungi were visible on about the 6th day and continued their vigorous growth till about the 20th day. In all the bottles *Coprinus* predominated and the mycelium was so intimately intertwined with the material as to form one solid mass. In the case of the material decomposed by soil micro-flora, the fungus mycelium disappeared after about 32 days but in this case, even after the completion of its vigorous growth, the mycelium retained this state during the rest of the period. The whole experimental period in this case as in previous experiments was 40 days. Two cases only showed bacterial infection and these were therefore omitted from consideration.

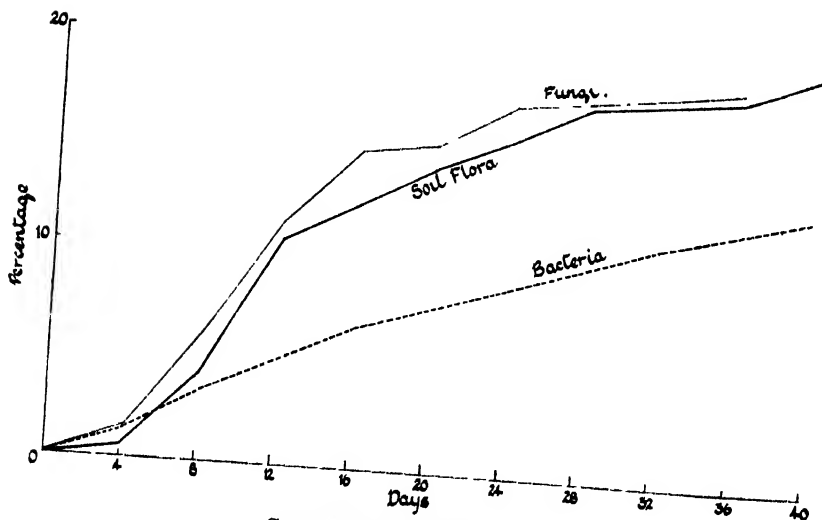
#### Series VII. Decomposition of rice-straw by bacteria.

This series was devoted to the study of bacterial activities in the decomposition of organic materials. As it was not possible to obtain all the bacteria from one kind of medium, the following media were used for their isolation: (1) Thornton's medium (37), (2) nutrient agar, (3) nitrate agar, (4) filter paper in mineral salt solution. As the micro-flora is shown to change (24) during different stages of decomposition of cellulosic materials, a mixture of fresh straw and decomposed manure was used as a source of these bacteria. The temperature of incubation was 35° C.

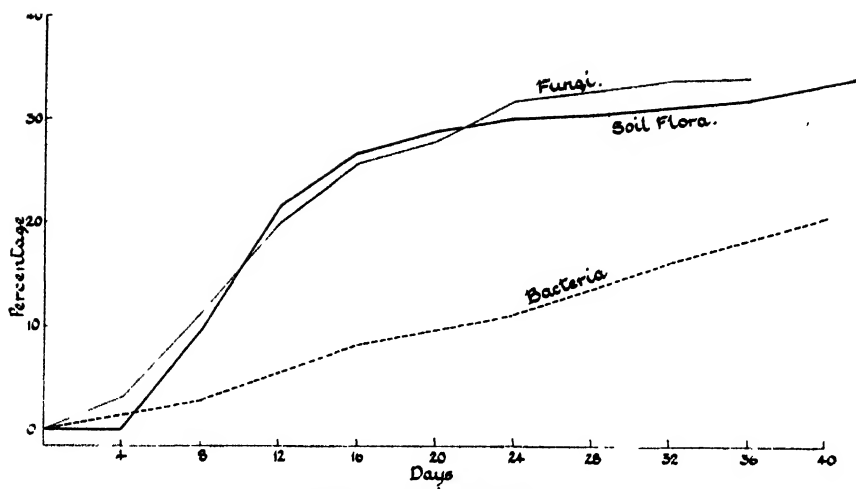
The same straw used in previous experiments was utilised in this case; but owing to the lack of sufficient quantity of this straw, the period for analysis was lengthened to 8 days. The methods of sterilisation and inoculation of this straw were the same as in Series VIII. Bacteria obtained on these media were used for inoculation without any attempt to identify them. In addition to the cellulose decomposing bacteria obtained on the filter paper medium, pure cultures of *Spirochaeta cytophaga* and *Microspira agar-liquefaciens* were also added. It was hoped that this mixture of bacteria would be a fair representation of all those active in the decomposition of organic materials. The decomposition in this case was very slow and the straw did not lose its tubular structure during the whole experimental period of 40 days.



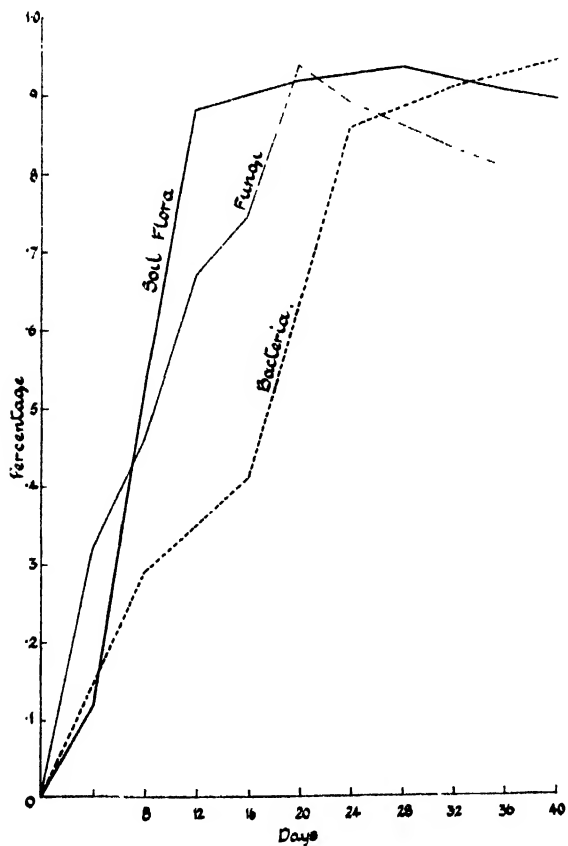
Graph 3. Loss of organic matter.



Graph 4. Loss of pentosans



Graph 5. Loss of cellulose.



Graph 6. Nitrogen factor.



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In all these series attention was specially directed to the quantitative estimation of the loss of organic matter, pentosans and cellulose. Incidentally the nitrogen-factor was also determined. The results are represented graphically.

### Series VIII. Nitrification tests.

In this series, the products of the activities of these micro-organisms (Series VII, VIII and IX) were subjected to nitrification tests. A quantity of 200 gm. of soil was used for each bottle and these various materials in finely powdered condition were added in equal amounts of 40 parts of nitrogen to a million parts of soil and thoroughly mixed. Moisture was kept at 60 per cent. of the saturation. A control was run with the same amount of soil at the same moisture content. All the bottles were incubated in a cellar where the temperature was about 12° to 15° C. In some cases the experiment was repeated with the soil of low nitrate content.

After an incubation of one month, analysis for nitrate was carried out by the method described by Russell and Page<sup>(25)</sup> in their methods for soil analysis. The method depends upon the oxidation of organic matter in the water extract of the soil by alkaline permanganate and determination of ammonia obtained by reduction of nitrate with Devarda alloy in the residual liquid. The results are calculated as nitrate parts per million and are given in Table VIII.

Table VIII.

Nitrate nitrogen—parts per million. Experimental period—one month.

Rice-straw inoculated with	Control soil only	Rice-straw decomposed for 16 days only	Rice-straw decomposed for 36 days only
I. Soil extract (Series VII)	(1) 53.7 (2) 19.1	36.8 13.1	55.7 20.1
II. Fungi (Series VIII)	(1) 53.7 (2) 19.1	52.9 18.9	55.6 19.8
III. Bacteria (Series IX)	(1) — (2) 19.1	— 8.8	— 11.0

In the case of bacteria straw, which had been decomposed for 40 days, was used.

*Discussion.* It is quite evident from the results (see Graphs 3, 4, 5), that, given the proper conditions, the combined fungi are as active in the decomposition of the organic materials as the whole soil micro-flora. It must be admitted that the preliminary conditions in both cases were not exactly similar. Firstly in the case of fungi, the straw was subjected

to the drastic treatment of sterilisation. Chemically this was found to affect only the cellulose which gave a yield about 4 per cent. less than in the unsterilised straw. It would be impossible to judge what physical changes it had brought about; but it has been generally assumed that autoclaving makes a plant material more susceptible to microbial attack. Secondly inoculation in this case was far in excess of what would happen under natural conditions. The difference between the coefficients of curves for the loss of organic matter in the case of fungi and soil micro-flora respectively is not significant. The loss of pentosans as well as cellulose gives further confirmation as both the curves for fungi run more or less closely parallel with those of soil flora (see Graphs 4, 5). Therefore, even after making allowances for differences of treatment in the two cases, it can be fairly assumed that fungi alone can do the whole work of decomposition almost as efficiently as the soil micro-flora.

The bacteria, on the whole, do not seem to be as active as fungi. In both these cases the condition of the medium was exactly similar. Inoculation in this case was also far in excess of what occurs under ordinary conditions. Though it is not claimed that all the bacteria active in the soil were obtained, different types of media used for their isolation would assure a fair representation of the prominent bacteria. A similar limitation was also working in the case of fungi as only three species were used in the experiment. It is therefore justifiable to assume that fungi show a far greater activity than bacteria in the decomposition of organic matter, and therefore under natural conditions they play a more important part in the decomposition of cellulosic materials.

Though comparison of the nitrogen factor (see Graph 6) leads more or less to the above conclusion, there is a striking dissimilarity at later stages between the three curves. It would seem that although fungi during their vigorous growth locked up soluble nitrogen supplied as ammonium carbonate, they began to ammonify some portion so absorbed. The curves therefore showed a rapid downward tendency, and had the experiments been continued after 40 days, the nitrogen factor would probably have been greatly reduced. In the case of bacteria quite the reverse phenomenon was observed during the whole experimental period. The locking of nitrogen was very slow but rose during the whole experimental period. The curve for the soil micro-flora seemed to be a balance between these two curves and might indicate a balance between the activities of these two groups of micro-organisms.

The nitrification tests brought out two important points. Firstly, they showed that the loss of organic matter was no proper index of the

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availability of nitrogen. Rice-straw had lost 45 per cent. of its organic matter during 36 days of its decomposition, but the nitrogen it had locked up during this process from the ammonium carbonate due to the activity of the micro-organisms did not seem to be easily nitrifiable. No doubt this decomposed material had no unfavourable effect on the nitrate already present in the soil, observed in the case of the straw which was decomposed only for 16 days. It could be assumed therefore that easily available carbohydrates which are generally considered to cause reduction in nitrates are broken down during those 36 days; but on the other hand the ammonium compound which was assimilated during this process by micro-organisms seemed to be transformed into some resistant complex which would be required to pass through some other stages of decomposition before being easily nitrified.

Secondly, fungi seemed to be more efficient in removing easily assimilable carbohydrates as the product after 16 days' decomposition in this case had not led to any loss of nitrate already present in the soil. The curves (see Graph 4) for the loss of pentosans indicated that fungi used more pentosans during the first 16 days than the total soil flora and this might be the reason of the big difference in the nitrate nitrogen figures in the two cases. The loss in the nitrate nitrogen in the case of bacteria even after 40 days could be easily explained by the slow decomposition the material had sustained during this period.

### Series IX. Pot culture tests.

In this series, pot culture tests were carried out on *Coprinus* to see whether its nitrogen was easily available to plant growth. To get a comparative evaluation, ammonium sulphate and dried blood were also tried.

*Coprinus* was grown on straw mixed with assimilable nitrogen. As the temperature of the growth was 35° C. there was much vegetative growth, all the fruiting bodies being sterile. It was separated from straw, dried and powdered. This separation from straw was quite easy as the fruiting bodies rising above the surface of the straw could be easily removed, but in some cases bits of straw remained attached to the fungus, and it was therefore assumed that the material thus obtained contained about 2 per cent. straw. Its nitrogen content was found to be 3.5 per cent. of dry weight.

Four pots were used for each set and the material was added to the soil as 55 parts of nitrogen to one million parts of soil. Mustard was grown in pots as it responds well to nitrogen. *Coprinus* pots showed

very good growth at the start, but at later stages they lagged behind, and at the time of cutting there was no significant difference between this set and the control. The plants in both sets showed early maturity, and were thin with less leaf area. The results of the wet and dry weight of the plants are given in the following table:

Table IX.

*Availability of nitrogen for plant growth.*

Average for four pots in each set.		
	Wet weight gm.	Dry weight gm.
Control	14.4	3.85
Ammonium sulphate	29.1	7.4
Dried blood	24.7	7.1
<i>Coprinus</i>	17.7	4.7

The results clearly show that the nitrogen in *Coprinus* is not in an easily available condition. This would very well explain the low nitrifiability of manures observed in Series VIII. During the 36 days period of the decomposition of the straw, the immobilised nitrogen may have been mostly in the fungus bodies.

(C). *A study of the behaviour of certain fungi which were found to play the more important part in the process.*

It is quite clear from the previous section that fungi are important agents in the decomposition of plant materials in the presence of assimilable nitrogen. They can go through the whole process necessary to produce rotted organic manures which are found by the nitrification test to be as good as those ordinarily produced by the activity of all the groups of micro-organisms present in the soil. It is therefore of considerable importance to study their behaviour in pure culture towards various food constituents, and in this section an examination is made of three fungi active in such decomposition.

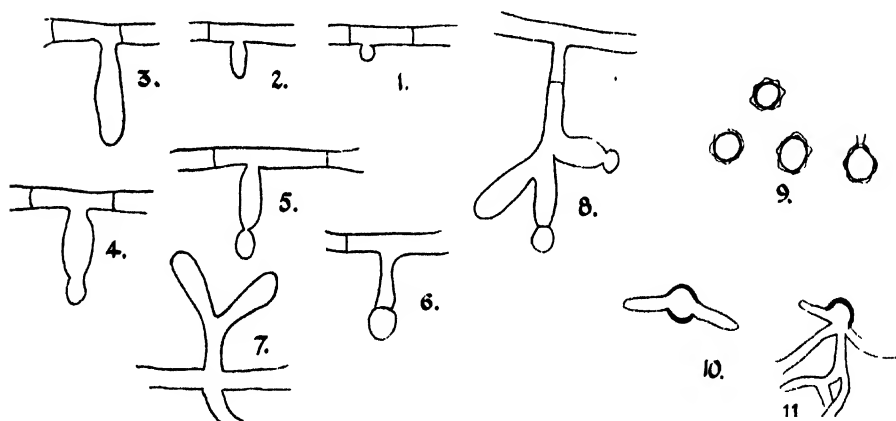
*Method of Isolation.*

Coons' agar acidified to  $pH = 4$  was used as a medium for isolating these fungi. The inoculum was prepared from a decomposing heap of wheat straw treated with assimilable compounds of nitrogen. Two temperatures were used for incubation, viz.  $35^{\circ}C.$  and  $50^{\circ}C.$ , the temperature of the decomposing heap being about the latter. Fungal growth was obtained on the medium at both the temperatures and this was found to be a mixture of fungi: further separation was done on potato agar.

*Morphological description.*

*Aspergillus* sp. (resembles most closely *A. fumigatus*).

*Colony on synthetic potato-agar* (5), pH 6.2, temperature 35° C., white in vegetative stage, green when sporulating, turning brown with age; strict, with scattered aerial hyphae, secondary growth; no coloration of the medium. Submerged and superficial sterile hyphae creeping, greatly branched, septate, hyaline. Margin of the colony spreading, transparent. Conidiophores unbranched (rarely forked in two), aseptate, arising directly from the substratum or as side branches; when from the substratum, 80 to 200  $\times$  6 to 10  $\mu$ , when as side branches 20 to 42  $\mu$  in length. The surface of the conidiophore is smooth, hyaline or scattered with granules. The conidiophore ends with a globose swelling, brownish in



Text-fig. 1. *Acremoniella* sp. (*velutina*?). 1-6. Development of spore. 7-8. Branched sporophores. 9. Mature spores. 10-11. Germination of spores. All magnified  $\times 600$ .

colour, 10 to 22  $\mu$  in diameter, closely beset with simple sterigmata, pointing forwards, very close, numerous, narrow at the tip 6 to 8  $\mu$  in length. Conidia oval, 2 to 4  $\mu$  in diameter, smooth, hyaline singly, green when young, brown when mature in mass, in long chains, forming dark cylindrical heads 58 to 90  $\times$  37 to 45  $\mu$ ; chain of spores broken up readily when mounted. Germination of the spores commonly by two tubes at opposite ends of the spores.

*Acremoniella* sp. (resembles most closely *A. velutina* (Fuck) except in the shape of the spores).

*Colony on synthetic potato-agar* (5), pH 6.2, temperature 50° C., white in

vegetative stage, green turning to black when sporulating. Sectoring of the colony is very common. Strict with scattered aerial hyphae. No secondary growth. Coloration of the medium between cinnamon and fawn colour Ridgway (30). Margin consisting of submerged vegetative hyphae. Hyphae complexly branched, hyaline, septate; no differentiation between vegetative and reproductive hyphae. Surface mycelium carrying short sporophores laterally, generally single, rarely branched in twos and threes, hyaline, septate or aseptate; occasionally two or more arise at the same place or on opposite sides forming a cluster, 15 to 32  $\mu$ . Spores single on each sporophore, oval when young, globose when fully developed, brown, thick-walled, with markings on the surface, 6 to 9  $\mu$ . The spore breaks from the sporophore, sometimes retaining a portion of the latter. Germination normally by two or more tubes from a single opening. (See Text-fig. 1.)

*Coprinus* sp. (resembles most closely *C. fimetarius* Fr.).

The morphological examination in this case was carried out on straw moistened with assimilable nitrogen at laboratory temperature (about 15° C.).

Cap, when young ovate, 2 to 3 cm. high when fully grown, margin unequal, then more or less expanded, companulate, at first even; during growth the cuticle gets torn into adpressed shaggy scales which can easily be removed and are white in colour, interstices grey, disc pale ochraceous, remaining entire, margin torn; stem white, 3 to 5 cm. long at ordinary temperature (15° C.), more (10 to 15 cm.) at 35° C., cylindrical, sub-equal or slightly upwards, fibrous, hollow, with a cord of filaments in the cavity, covered with silky hairs which soon fall off, even, bulbous at the base, bulb solid, ring absent; gills free, distant from the stem, white then blackish; spores black, elliptical, 9 to 12  $\times$  7 to 9  $\mu$ . germination by two or more tubes.

### PHYSIOLOGICAL STUDIES.

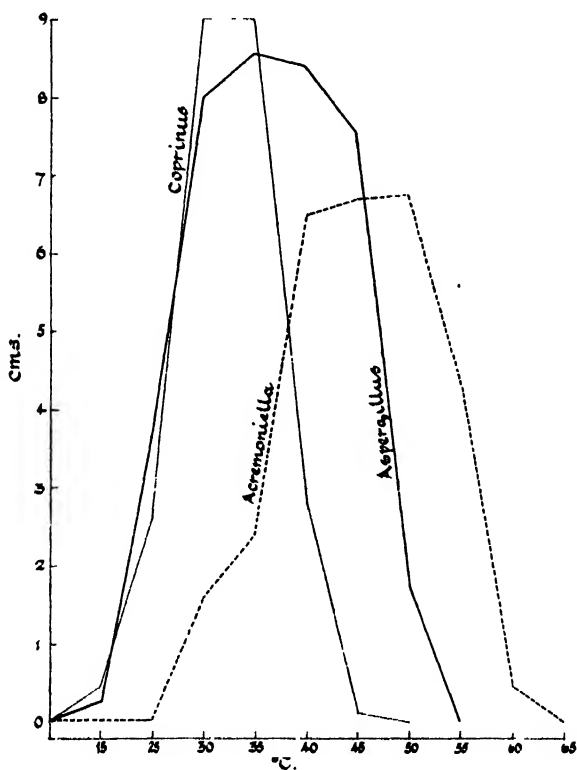
#### 1. *Temperature relationship.*

The growth of these fungi at various temperatures was studied on potato-agar in petri-dishes. Four petri-dishes, containing the same amount of medium (20 c.c.), were utilised for every temperature, and a definite quantity of inoculum of spores in sterile tap water (1 mm. loop) was placed in the centre of each petri-dish. They were then placed in columns face downwards in moist containers, a precaution taken to prevent drying of the medium at high temperatures. Generally the

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incubation period was only for six days, but where the growth was slow it was extended to 12 and 24 days.

Measurements of the growth were made in every case on the 3rd, 6th, 12th and 24th day in two directions at right angles to each other, each measurement being made to the nearest half-millimetre. In cases where the colony did not grow circularly, measurements were made along the long and short diameter and the average taken. In the case of *Coprinus* sp. measurements were taken only on the 6th day.



Graph 7. Six days' growth of fungi at different temperatures.

The stock cultures which supplied the inocula were grown on synthetic potato-agar at 35° C. in the case of *Aspergillus* and *Acremoniella*. The *Coprinus* was grown on straw at ordinary temperature and the spores were collected on sterile cover-slips at the time of their shedding. Owing to the difficulty of obtaining a uniform suspension of spores as a result of their impermeability, especially in the case of *Aspergillus* sp., the inoculum

in each case was likely to differ in the number of spores. A count of 10 such inocula showed that the number of spores varies from 22 to 57. The actual measurement of growth in the petri-dishes showed that this big variation had no significant effect on the growth of the colony and therefore the quantitative measurements are sufficiently reliable. The results are represented in Graph 7.

## 2. *Growth on media containing carbon and nitrogen compounds.*

The mineral salt solution used as a basis for the study of the behaviour of these fungi towards different carbon and nitrogen compounds had the following composition:

MgSO <sub>4</sub> .7H <sub>2</sub> O	...	...	...	0.5 gm.
K <sub>2</sub> HSO <sub>4</sub>	...	...	...	1.0 "
KCl	.	..	...	0.5 "
FeSO <sub>4</sub>	...	.	..	0.01 "
Water	.	..	.	1000 c.c.

Compounds of nitrogen were added at the rate of 2 gm. per 1000 c.c. of the medium and include peptone, ammonium sulphate, sodium nitrate and asparagin. The carbon compounds were used at the rate of 1 per cent. and consisted of dextrose, saccharose, maltose, xylose, arabinose, galactose, mannose, starch, straw gum, cellulose and lignin. All the media contained agar as 15 gm. per 1000 c.c.

Cellulose for the medium was obtained by dissolving filter paper in Schweitzer's reagent and precipitating it with acid (18). In addition to the cellulose agar medium, filter paper strips in mineral salt solution were also tried for the fungus growth. Lignin was extracted from wheat-straw by the method of Beckman, Liesche and Lehman (2).

The study of the behaviour of these fungi on the various media was done in petri-dishes. The amount of inoculum as well as the method of inoculation was the same as described in temperature relationship. The petri-dishes were incubated at the optimum temperature for the growth of these fungi, viz. 35° C. in the case of *Aspergillus* and *Coprinus* and 50° C. for *Acremoniella*. In the case of the latter, additional petri-dishes were incubated at 35° C. for certain media in which the fungus did not show good growth at the higher temperature. The period of incubation was generally 12 days.

The petri-dish cultures were made in order to take quantitative measurements of growth; but it was soon found that though this method was fairly accurate for one medium under different conditions, it was



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Table X.

*Behaviour of fungi on pure nitrogen and carbon compounds.*

	35° C. <i>Aspergillus</i> sp.*				50° C. <i>Acroniella</i> sp.			35° C. <i>Coprinus</i> sp.†
Period 12 dyas	(growth		Sporu- lation	Growth	Sporu- lation	Colour of the medium		Growth
Nitrate + (carbon compounds)								
Glucose ... ..	...	...	×	×	×	×	×	×
Saccharose ... ..	...	...	×	×	×	×	×	×
Maltose ... ..	...	...	×	×	×	×	×	×
Xylose ... ..	...	...	×	×	×	×	×	×
Arabinose ... ..	...	...	×	×	×	×	×	×
Galactose ... ..	...	...	×	×	×	×	×	×
Mannose ... ..	...	...	×	×	×	×	×	×
Starch ... ..	...	...	×	×	×	×	×	×
Cellulose ... ..	...	...	×	×	×	×	×	×
Straw gum ... ..	...	...	×	×	×	×	×	×
Lignin ... ..	...	...	Nil	Nil	} growth in all cases poor, very little sporulation			
Peptone + (carbon compounds)								
Glucose ... ..	...	...	×	×	×	×	Nil	×
Saccharose ... ..	...	...	×	×	×	×	Between cinnamon (XXIX) and fawn colour (XL)	×
Maltose ... ..	...	...	—	—	×	×	Nil	×
Xylose ... ..	...	...	×	×	×	×	"	—
Arabinose ... ..	...	...	×	×	×	×	"	—
Galactose ... ..	...	...	—	—	×	×	"	×
Mannose ... ..	...	...	—	—	×	×	Same as saccharose	×
Starch ... ..	...	...	×	×	×	×	Nil	×
Cellulose ... ..	...	...	—	—	Nil	Nil	"	Nil
Straw gum ... ..	...	...	—	—	×	×	"	×
Lignin ... ..	...	...	—	—	×	×	"	×
Ammonium sulphate + glucose	...	...	×	×	×	—	"	—
Asparagin + glucose	...	...	×	×	×	×	"	×
Casein + glucose	...	...	×	×	×	×	Varies between i and l (XXVIII)	×
Egg-albumen + glucose	...	...	×	×	×	×	Cameo brown (XXVIII) l	×
Potato agar ... ..	...	...	×	×	×	×	Same as saccharose	×
Synthetic potato agar ...	...	...	×	×	×	×	Nil	×
Straw extract agar ... ..	...	...	×	×	×	×	"	—
Prune extract agar ... ..	...	...	×	×	×	Nil	"	/
Filter paper in mineral salt ...	...	...	×	×	Nil	"	"	Nil
Raulin's solution ... ..	...	...	×	×	"	"	"	×
White of egg (liquefying power)	...	...	×	×	×	×	Colour same as egg albumen agar. No liquefaction	×
	...	...	Nil					Nil

\* *Aspergillus*, no colour in the medium.

† *Coprinus*, no colour in the medium, no sporulation.

N.B. Period of incubation—12 days. Ridgway's *Colour Standards and Nomenclature*.

The symbol × × × × represents the best growth as well as the best sporulation, while the symbol / represents germination in the case of the growth and visibility of the spores under the microscope in the case of sporulation. The others represent the intermediate stages.

quite unsuitable for different media owing to the variation in the type of growth of the one fungus. By this system thin spreading growth gives high quantitative results even though the visual inspection would show quite the reverse. Also it does not take sporulation into account. For these reasons quantitative measurements were discontinued in all these cases. A qualitative idea is obtained by the visual inspection of

the growth on three petri-dishes in each case. This is symbolically represented in Table X. The symbol  $\times \times \times$  represents the best growth and the symbol / only the germination of spores, the others representing intermediate stages. The symbol / in the case of sporulation indicates that the spores are visible under the microscope only.

In addition to the above media, the following standard media were also tried: prune extract agar, straw extract agar, Raulin's solution, synthetic potato agar<sup>(5)</sup>, casein glucose agar, potato agar, egg-albumen glucose agar. The liquefaction power of these fungi was tested on white-of-egg. The usual procedure of utilising gelatine for such an experiment could not be adopted owing to the high temperature necessary for this study.

### 3. Study of enzymic activity.

A few tests of the enzymic activity of these fungi were carried out according to Crabill and Reed's methods<sup>(6)</sup>, and the results are shown in Table XI.

Nitrogen was added to the stock medium in the form of nitrate and peptone. As can be seen from Table X, while nitrate is favourable for the growth of *Aspergillus* and *Coprinus* it is inhibitive to the growth of *Acremoniella*. On the other hand *Aspergillus* prefers nitrate to peptone which is most suitable for the growth of *Acremoniella*. Thus for the study of each enzyme it was necessary to use two media varying in the type of nitrogenous compounds.

### 4. Action on cellulosic materials.

Rice-straw was chosen as a cellulosic material for this study. It was sterilised in bottles and inoculated with the pure cultures of these fungi singly as well as in all possible combinations. Method of sterilisation, addition of ammonium carbonate and inoculation of these fungi was exactly the same as described in Section B, Series II.

All the bottles were inoculated at 35° C. In the case of *Acremoniella* an additional bottle was incubated at 50° C. which is the optimum temperature for its activity. The experiment was stopped after 36 days and the contents were analysed. The results are given in Table XII.

*Acremoniella* did not show any activity at either temperature, while *Aspergillus* took nearly a fortnight for a start. In the case of the latter, sporulation was the prominent feature. Though *Coprinus* showed early growth, the decomposition was very slow even in this case. The various combinations of these fungi in twos worked better than the individual fungus; but all three together seemed best for the decomposition.

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Table XI.

*Enzymic activity of fungi.*

<i>Aspergillus</i> sp.					
Enzymes	Medium	Growth	Dissolution of the particles	Halo	Reaction
Erepsin	Casein agar	Fair	Slight	Fair	--
Trypsin	Egg-albumen agar	"	Good	None	---
Amidase	Asparagin-rosolic acid agar	Good	—	—	Deep brilliant red, widely diffused
Cytase	Straw gum agar	Fair	Doubtful	None	--
Cellulase	Cellulose agar	Good	None	"	---
Amylase	Starch agar	Very good	—	—	Yellow
Lignin-decomposing enzyme	Lignin agar	None	None	None	—
<i>Acremonella</i> sp.					
Erepsin	Casein agar	Poor	Fair	None	---
Trypsin	Egg-albumen agar	Good	Good	"	---
Amidase	Asparagin-rosolic acid agar	Fair	—	—	Slight red
Cytase	Straw gum agar	Good	Slight	None	---
Cellulase	Cellulose agar	None	None	"	---
Amylase	Starch agar	Very good	—	—	Yellow
Lignin-decomposing enzyme	Lignin agar	Fair	Doubtful	None	---
<i>Coprinus</i> sp.					
Erepsin	Casein agar	Good	Good	None	---
Trypsin	Egg-albumen agar	Very good	Very good	"	---
Amidase	Asparagin-rosolic acid agar	Good	—	—	Deep red diffused
Cytase	Straw gum agar	Slight	Doubtful	None	--
Cellulase	Cellulose agar	None	None	"	---
Amylase	Starch agar	Very good	—	—	Yellow
Lignin-decomposing enzyme	Lignin agar	Slight	Doubtful	None	—

Table XII.

*Decomposition of rice-straw by fungi.*

Calculated on 100 gm. of dry matter.

	Loss of dry matter	Nitrogen factor
<i>Coprinus</i>	23.2	0.5
<i>Coprinus</i> + <i>Aspergillus</i>	27.2	0.51
<i>Coprinus</i> + <i>Acremoniella</i>	26.3	0.43
<i>Aspergillus</i>	16.0	0.54
<i>Aspergillus</i> + <i>Acremoniella</i>	28.0	0.34
<i>Acremoniella</i>	0.0	—
<i>Aspergillus</i> + <i>Acremoniella</i> + <i>Coprinus</i>	40.6	0.76

5. *Physiological characteristics of individual fungus.**Aspergillus* sp.

The optimum temperature for the *Aspergillus* seems to lie between 30° and 40° C. and the maximum at about 50° C. Its growth below 30° C. is very slow and at the ordinary temperature of the laboratory, which was about 15° C., the spores took about 12 days to germinate. Thus this fungus cannot be active in the decomposition of cellulosic materials at a temperature below 30° C.

Table X indicates that the *Aspergillus* has no special affinity for any one carbohydrate. Though there is a slight variation of growth on the different media, it is not significant to require special consideration. It shows moderately good growth even on cellulose media. On the other hand it shows poor growth on straw gum, which cannot therefore act as a substitute for its hydrolytic products—xylose and others. This might be the reason for the slow activity of the fungus on rice-straw, as the lack of this hydrolysing enzyme would naturally prevent it from making use of the easily available food material—the pentosans.

Among the nitrogen compounds, nitrate comes the first, though in some carbohydrate media, peptone is as good as nitrate. Asparagin comes next, but ammonium sulphate supplies poor nourishment. As this fungus shows the best growth on an acid medium (pH = 4.6) this inhibitory effect of ammonium sulphate may not be due to acidity.

The colour of the spores seems to be dependent upon the nature of the carbohydrates. While dextrose and saccharose cause the production of dark green spores, the fungus on arabinose and starch has spores of a paler tinge. Further, the browning of the spores observed in the case of growths on potato agar about the 6th day, was not visible in the case of growths on all these carbohydrates even on the 12th day. A study of Table XI indicates that the predominance of sporulation over the vegetative stage is due to the poverty of the medium in the requisite food ingredients. Cellulose agar or white-of-egg are good examples. On the other hand, rich media such as Raulin's solution brings about quite the reverse effect.

*Acremoniella* sp.

The optimum temperature for the growth of *Acremoniella* lies between 40° and 50° C. and the maximum at about 60° C. Though isolated spores do not germinate at higher temperatures, they are found to germinate up to about 70° C. if inoculated in agglutinated mass. The spores are not found to germinate at all below 25° C. The necessity of

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high temperature for its growth indicates that ordinarily this organism cannot be active in the decomposition of cellulosic materials. But during the first stages of the decomposition of manure heaps, the temperature goes as high as 55° C. and sometimes even higher. Generally speaking thermophilic bacteria are considered responsible for the decomposition during this period; but it seems reasonable to assume that this fungus can also take part during that process.

Though the fungus requires high temperature for its growth, it seems to be susceptible to sudden changes of temperature. Thus it is found that an inoculum from cultures grown at 50° C. and kept afterwards at room temperature for more than a month will not grow if incubated at its optimum temperature of 50° C. It is necessary either to raise it gradually from lower temperatures (35° C.) upwards or to inoculate the spores in agglutinated mass if incubated at once at the high temperature. Even in the latter case one or two transfers are necessary for it to regain its original vitality. Whether it would show the same phenomenon under the natural condition of its activity is not known. But this characteristic is repeatedly observed on artificial media. Sectoring occurs frequently in cultures of this fungus and is probably the basis of its change in temperature relationships.

This fungus shows great diversity in growth on different nitrogen and carbon compounds. Nitrate and ammonium sulphate have an inhibitory effect. Not only the growth is poor, but the hyphae are closely felted together and in some cases as in nitrate saccharose agar, they showed beaded appearance. Sporulation is also generally absent. In some cases sporophores can be seen under the microscope with immature spores. Peptone is the best nitrogenous food, asparagin coming next to it.

Among the carbon compounds, pentoses seem to supply poor nutrients to this fungus; but it grows very well on straw gum. Perhaps the concentration of these sugars might be too high for it. In the case of straw gum, insolubility of the gum would not lead to the increase in the concentration. It does not show any growth at all on cellulose in solid as well as liquid media. On lignin some growth is made though the fungus is not vigorous on it. In other carbohydrates, *Acremoniella* shows vigorous growth and in such cases, sectoring is common.

The general colour of the colony is grey though individual spores are dark brown. The spores, in contrast to those of *Aspergillus*, remain moist in all the media tested. They are not therefore easily scattered by the wind as is the case with the *Aspergillus* spores. *Acremoniella*

also produces colour in the medium which is in certain cases, as on egg medium, very characteristic.

*Coprinus* sp.

The optimum temperature for the growth of *Coprinus* lies between 30° and 35° C. and the maximum at about 45° C. In this case, the best temperature for growth is not the best for spore production as almost all the fruiting bodies are sterile. The fruiting bodies grow very vigorously, reaching a height of 8 inches on straw and also branch copiously; but the cap is almost always very small, hard and yellow, without scales, and sterile. At this temperature only in rare cases was it found to give fertile fruiting bodies. But sclerotia—10 to 15 brown irregularly shaped bodies—are quite common both on the media as well as on the straw. At ordinary temperature as well as at 25° C. growth is very slow, the maximum height to which the fruiting body is found to reach being 3 inches. But generally all the fruiting bodies are fertile. The shedded spores kept on sterile cover-slips are found to germinate even after six months.

Except galactose and mannose, all the carbohydrates are suited to its growth. The most vigorous growth is found on glucose and maltose, where the colony not only spreads even outside the petri-dish due to the fluffy growth, but is full of sclerotia.

## V. GENERAL DISCUSSION AND CONCLUSIONS.

In the discussion of this work, it must be borne in mind that plant materials containing large quantities of hexoses and starches are generally used as food either for animal or man and are not therefore likely to be utilised as waste for conversion into manure. Thus the materials available for manurial purposes are deficient in both these constituents. However, the process of rotting does not appear to be unfavourably influenced by the elimination of these constituents and it can therefore be assumed that they have no important bearing on the decomposition of these materials.

As regards cellulose, this forms a major portion of the fundamental plant tissue and is of importance in the decomposition. Perhaps the greatest difficulty in tracing the behaviour of cellulose is the lack of any reliable method for its estimation. Out of all the methods so far proposed, the chlorination method of Cross and Bevan<sup>(8)</sup> is generally considered to be the best; even by this method, however, not only does the preliminary alkali treatment lead to a slight loss of cellulose, but the

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chlorine after first combining with lignin starts to attack the cellulose itself. As the decomposition by organisms proceeds, therefore, the surface area of the cellulose is increased which makes it more susceptible to the chemical treatment. This is confirmed by Graph 5 which shows that the loss of cellulose from the rice-straw decomposed by fungi alone is very rapid though only one of these fungi is found to grow slightly on pure cellulose. The apparent large loss of cellulose is therefore not purely due to the microbial activity and would not give an index as to its food value to the micro-organisms. This must be borne in mind in seeking to interpret any analytical data in connection with cellulose decomposition. Thus failing any reliable direct evidence it is necessary in order to obtain an idea of the importance of cellulose to use indirect means. This has been made possible by the physiological studies of the organisms most active in the rotting of this type. An investigation into the relative importance of bacteria and fungi in these decompositions shows the latter to be far more active than the former. The behaviour of these active fungi towards different purified carbon compounds shows that pure cellulose is itself a poor nourishment especially at the start, and it seems, therefore, that though cellulose may be easily decomposed once the organisms are active, it would not be a factor controlling the "decomposibility" of the material.

Investigations by many workers have proved that lignin is very resistant to microbial attack. Except certain timber rotting fungi such as *Trametes pini*, etc., which do not commonly occur either in soil or manure, organisms do not flourish solely on lignin. The physiological studies here reported of certain fungi acting in such decompositions also show that, except in one doubtful case, lignin does not sustain their growth, and it is therefore quite natural to assume that lignin would not only be a poor nourishment to micro-organisms, but being a part of the fundamental tissue would, on account of its resistant nature, act as a physical barrier, thus hindering their ability to get their food. If this be true the high lignin content of any cellulosic matter would be detrimental to its decomposition and this is found to be the case with many of the materials.

Next to cellulose, hemi-cellulose forms an important constituent of plant tissue; but like cellulose, this is not a chemical entity as on hydrolysis it not only yields separate distinct compounds, but these compounds vary in quantity in different cellulosic materials. Thus its estimation, as a whole, does not lead one to any definite conclusions. The only possible way, therefore, of getting an idea of the value of these

various products of hydrolysis in the process of decomposition is to study their effect on the activity of micro-organisms. It has been shown (Series IV) that both galactose and mannose offer poor nourishment to the organisms active in such decompositions. The physiological studies of fungi also confirm their poverty as nourishment to the most active fungus (*Coprinus*). On the other hand, both by its rapid disappearance during the process of decomposition, as well as by the nutritive value of the products of hydrolysis, the pentosan part of the hemi-celluloses is found to supply the best energy to these micro-organisms. Pentosans form the larger part of the material which is attacked at the start, and though the loss in other constituents at later stages masks their importance, they are found to be essential to start the process of decomposition. The predominance of fungi in such decompositions further supports this assumption as studies on their physiology show that pentosans either in their natural state or in hydrolysed form supply the best nutrition to these organisms. This also lends confirmation to the conclusions of various workers who consider pentosans as the most easily decomposable constituent.

The study of the decomposition of the carbonaceous constituents of plant materials leads one, therefore, to conclude that pentosans form the important microbial food, and they may thus be assumed to control the "decomposibility" of a material. Further investigation on varied materials to test the validity of this hypothesis resulted in the knowledge of the unreliable nature of the analytical method for the determination of pentosans. It was found that the Krober and Tollens<sup>(1)</sup> method for pentosan determination included also some part of cellulose which was found both by direct fermentation tests as well as by indirect analytical estimation to be resistant to microbial attack. The apparent large figure for pentosans observed in the case of certain materials, such as poplar wood, consisted largely of this resistant compound and was therefore very misleading. The method had therefore to be modified to get as far as possible an accurate figure for pentosans. This was achieved by deducting the furfuroids yielded by cellulose from the total furfuroids, considering the remainder as representing pentosans. Even in this case certain exceptions to this hypothesis were found, thus showing that there was some other constituent besides pentosans which had a bearing on the process of decomposition. As discussed above, lignin could be taken as such a compound. Since it is of resistant nature, it would be expected to act as a physical barrier to the microbial search for food materials and therefore a balance between the two factors—



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nutritive or chemical and physical—would be likely to control the process of rotting. The application of this modified hypothesis in evaluating the results obtained (Table V) proved its validity; we are thus enabled to predict the “decomposibility” of a material by purely chemical analysis.

It is evident that the important condition for rapid decomposition of a material is the predominance of pentosans over lignin. This is found generally to be the case with the easily decomposable materials such as straws. On the other hand, woods consist of a large amount of lignin and are found to be very resistant. This indication of the basic principles underlying the process of decomposition naturally leads to the next step for its practical application, viz. the artificial stimulation of the process. There seemed to be two possible ways by which this could be achieved, either by a reduction of the lignin content or by an increase in the amount of pentosans. In the former case, it is essential to get an organism with special predilections for lignin; but among the micro-organisms active in such decompositions none is so far found to show such specificity. The second alternative was also found impracticable as organisms getting both their nitrogenous and carbonaceous food outside the tissues produced less decomposition of plant materials than was normally possible.

As regards the relative importance of the two groups of micro-organisms active in such processes, the present investigation proves that fungi are more important than bacteria, especially during the early stages of decomposition. Further, fungi are found capable of carrying out the whole process of breaking down of the cellulosic material as efficiently as the total soil micro-flora. But it seems that the nitrogen which these fungi immobilise in their body protein is not easily available for plant growth (see Series IX). It is therefore necessary that these bodies in turn must be decomposed by other organisms and the disappearance of fungal hyphae at later stages of decomposition suggests that nitrogen passes through the bodies of various organisms before it can be easily nitrifiable for plant growth.

In addition to the physiological peculiarities noted above, the study of these fungi shows a further interesting point. All three are most active at temperatures far above those usual for fungal growth and it would be an important point to note whether they themselves can bring about the rise in temperature necessary for their activity. A preliminary experiment in thermos flasks, when daily temperature records were taken of the straw which was *sterilised* and inoculated, very strongly suggests

this possibility, but further and more elaborate investigation is necessary before any definite conclusions can be carried out.

## VI. SUMMARY.

In mature plant materials pentosans form the most important food for micro-organisms.

The Klobber and Tollens method for the determination of pentosans is not specific for these compounds.

While pentosans are easily attacked by micro-organisms, the other furfural-yielding compounds are found to be resistant, and it is therefore essential to get a correct figure for pentosans. A possible method is suggested: to determine the furfuroids in the cellulose obtained by the chlorination method and to deduct this amount from the total furfuroids.

Two factors appear to control the decomposition of ripe cellulosic materials in the presence of assimilable nitrogen. The one is the food, or, better termed, *energy factor* which is the pentosans, the other is the physical or *inhibitory factor* which is the lignin. It is found that if the ratio of energy factor to inhibitory factor is above 1, the material is easily decomposed; but if it is below 0.5, the material is very resistant to microbial attack. The prediction of the "decomposability" of a material is thus possible.

Attempts to increase this ratio in resistant materials by the addition of carbohydrates proved unsuccessful. It was concluded that since micro-organisms obtained their food materials outside the tissues, they did not attack the tissues until the more easily available food-stuffs were exhausted. Thus the decomposition of the material was actually less than was possible under natural conditions.

Mannose and galactose do not appear to form suitable food for the micro-organisms concerned in these processes and it is concluded that the pentosan part of the hemi-celluloses is most important as microbial food.

The study of the relative importance of bacteria and fungi proves that under the conditions of these experiments, fungi play a more prominent part especially during the early stages of such decomposition.

The study on the availability of the nitrogen of the fungal bodies proves it to be of the resistant type. It seems that at later stages of decomposition under natural conditions fungi are decomposed by other organisms.

The ability of certain fungi isolated from such decomposing heaps, to grow at high temperature as well as on purified carbon constituents

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of plants, and also the presence of almost all the enzymes necessary to hydrolyse the complex carbon constituents, further confirm their importance. The possibility of their activity under natural conditions in manure heaps is strongly suggested.

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### VII. REFERENCES TO LITERATURE.

- (1) *A.O.A.C. Official and tentative methods for analysis*. 2nd edition.
- (2) BECKMAN, E., LIESCHE, O. and LEHMAN, F. (1921). *Z. Angew. Chem.* xxxiv, 285.
- (3) BRAY, M. W. and STAIDAL, J. A. (1922). *J. Ind. Eng. Ch.* xiv, 35.
- (4) BRAY, M. W. and ANDREWS, T. M. (1924). *Ibid.* xvi, 137.
- (5) BROWN, W. (1925). *Annals of Botany*, xxxix, 373.
- (6) CRABILL, C. H. and REED, H. S. (1915). *Biochemical Bulletin*, iv, No. 13, 30.
- (7) CROSS, C. F., BEVAN, E. J. and SMITH, C. (1897). *J.C.S.T.* 1001.
- (8) CROSS, C. F. and BEVAN, E. J. (1918). *Cellulose*.
- (9) DAVIS, W. A. and DAISH, A. J. (1914). *J. Agric. Sci.* vi, 152.
- (10) DVORAK, J. (1912). *Zeitsch. Landw. Vers. in Deutsch. Oester.* xv, 1077.
- (11) DORE, W. H. (1919). *J. Ind. Eng. Ch.* xi, 556.
- (12) DU TOIT, M. S. (1924). Thesis submitted to the Cambridge University for the Degree of Doctor of Philosophy.
- (13) HEBERT, A. (1892). *Ann. Agronomique*, xviii, 536.
- (14) HEUSER, E. and HAUG, R. (1918). *Z. Angew. Chem.* xxxi, 168.
- (15) HEUSER, E. and BOEDEKER, E. (1921). *J. Ind. Eng. Ch.* xxxiv, 461.
- (16) HUTCHINSON, H. B. and RICHARDS, E. H. (1921). *J. Min. Agr.* xxviii, 398.
- (17) JOHNSEN, B. and HOVEY, R. W. (1918). *Paper* xxi, 46.
- (18) KELLERMAN, K. F. and MACBETH, I. G. (1912). *Centralblatt f. Bakt. Abt. 34*, ii, 485.

- (19) LISSE, M. (1917). *J. Ind. Eng. Ch.* IX, 285.
- (20) MAHOOD, S. A. and CABLE (1920). *Paper* xxv, 11.
- (21) MEHTA, M. M. (1925). *Biochemical Journ.* XIX, 958.
- (22) MIGULA, W. (1913). *Forstw. Centbl.* N.S. XXXV, 161.
- (23) MITSCHERLICH, E. (1850). *Monatsber. d. K. Press. Akad. d. Wiss. zu Berlin*, 102 (quoted by MACBETH and SCALES in *U.S. Dept. Agr. Bur. Plant. Ind. Bul.* 1913, 266).
- (24) MURRAY, T. J. (1915-16). *Virginia Agr. Exp. Sta. Ann. Reports*, 103.
- (25) RUSSELL, E. J. and PAGE, H. J. (1925). *Chemist's Year Book*.
- (26) PELTIER, G. L. (1920). *J. Agric. Sci.* XX, 448.
- (27) RAHN, O. (1919). *Zeitsch. f. Tech. Biol.* VII, 172.
- (28) RICHARDS, E. H. and HUTCHINSON, H. B. (1924). *E.P.* 219, 384.
- (29) RICHARDS, E. H. and AMOORE, R. L. (1926). *Synthetic Farmyard Manure, Harpenden*.
- (30) RIDGWAY, R. (1912). *Colour Standards and Nomenclature*.
- (31) SACHS, J. (1882). *A text-book of Botany*. 2nd edition.
- (32) SCHULZE, E. and TOLLENS, B. (1892). *Ann.* CCLXXI, 55.
- (33) SIEBER, R. and WALTER, L. E. (1913). *Paper* XI, 1179.
- (34) SODERBAUM, H. G. and BARTHEL, C. (1924). *Bakteriologiska Avdelningen Ner. 36, Centralanstallern for Forsoksvasendet pa Jordbruksomradet*.
- (35) STARKEY, R. L. (1924). *Soil Sci.* XVII, 293.
- (36) THAYSEN, A. C. and BUNKER, H. J. (1923). *J. Roy. Micros. Soc.* 303.
- (37) THORNTON, H. G. (1922). *Ann. Appl. Biol.* IX, 241.
- (38) TOLLENS, B. and WHEELER, H. J. (1891). *Landw. Vers. Stat.* XXXIX, 436.
- (39) VAN SUCHTELEN, F. H. H. (1910). *Centr. Bakt.* XXVIII, ii, 45.
- (40) WAKSMAN, S. A. (1916). *Soil Sci.* II, 102.
- (41) WILLSTATTER, R. and ZECHMEISTER, L. (1913). *Ber. der Deutschen Chem. Gesellschaft*, XLVI, 2401.
- (42) WINTERSTEIN, E. (1893). *Z. Physiol. Chem.* XVII, 381.
- (43) WISLICENUS, H. and KLEINSTÜCK, M. (1910). *Z. Chem. Ind. Kolloide*, VII, 87.
- (44) WOLLNY, E. (1897). *Die Zersetzung der Organischen Stoffe und die Humusbildungen mit Rücksicht auf die Bodencultur*, 1897, Carl Winter, Heidelberg. Also in *Ann. de la Science Agron.* 1898, II, 339; 1899, I, 208; II, 260, 362; 1900, I, 1; II, 33, 338.

## EXPLANATION OF PLATES I—IV.

## PLATE I.

- Fig. 1. *Coprinus* sp. (*fmelarius*?) growing on sterilised oat-straw at laboratory temperature. Fertile sporophores are formed.
- Fig. 2. *Coprinus* sp. (*fmelarius*?) growing on sterilised rice-straw at 35° C. Sporophores are formed which frequently push the cotton-wool plug out of the neck of the bottle and emerge several inches. The sporophores are aborted and sterile.

## PLATE II.

- Fig. 1. *Acremoniella* sp. (*velutina*?). Six days' growth on Czapek's agar. At 25° C. and 65° C. the spores have germinated but are not visible in the photograph.
- Fig. 2. *Aspergillus* sp. (*fumigatus*?). Six days' growth on Czapek's agar. At 15° C. and 55° C. the spores have germinated but are not visible in the photograph.
- Fig. 3. *Coprinus* sp. (*fmelarius*?). Six days' growth on Czapek's agar. At 15° C. and 45° C. the growth is just visible. At 10° C. and 50° C. germination occurs but no growth is visible in the photograph.

## PLATE III.

- Fig. 1. *Acremoniella* sp. (*velutina*?). Growth on peptone medium + arabinose.
- Fig. 2.           "           "           "           "           + xylose.
- Fig. 3.           "           "           "           "           + mannose.
- Fig. 4.           "           "           "           "           + maltose.
- Fig. 5.           "           "           "           "           + saccharose.
- Fig. 6.           "           "           "           "           + lignin.
- Fig. 7.           "           "           "           "           + straw gum.
- Fig. 8.           "           "           "           casein medium.
- Fig. 9.           "           "           "           egg medium.

Fig. 3 shows sectoring of the colony.

## PLATE IV.

- Fig. 1. *Aspergillus* sp. (*fumigatus*?). Growth on glucose medium + nitrate.
- Fig. 2.           "           "           "           "           + peptone.
- Fig. 3.           "           "           "           "           + ammonia.
- Fig. 4.           "           "           "           "           + asparagin.
- Fig. 5. *Acremoniella* sp. (*velutina*?).           "           "           + nitrate.
- Fig. 6.           "           "           "           "           + peptone.
- Fig. 7.           "           "           "           "           + ammonia.
- Fig. 8.           "           "           "           "           + asparagin.

(Received July 19th, 1926.)

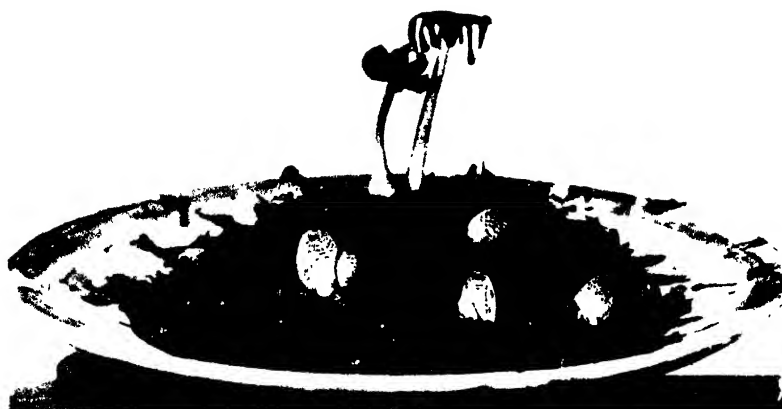


Fig. 1.



Fig. 2.





Fig. 1.



Fig. 2.

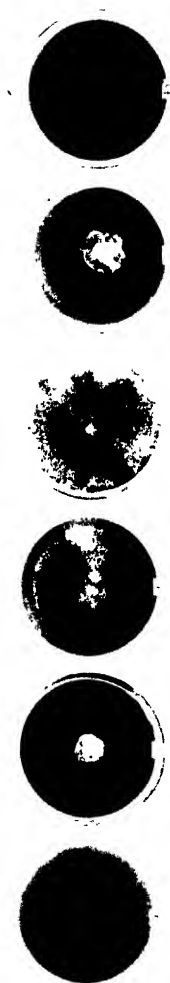


Fig. 3.





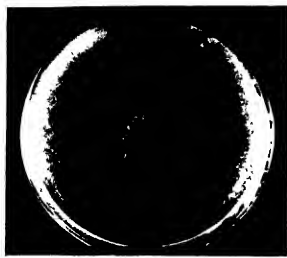


Fig. 8.

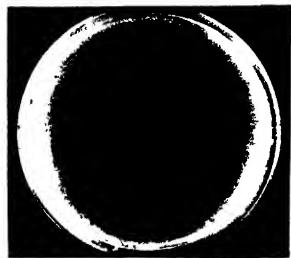


Fig. 9.



Fig. 7.

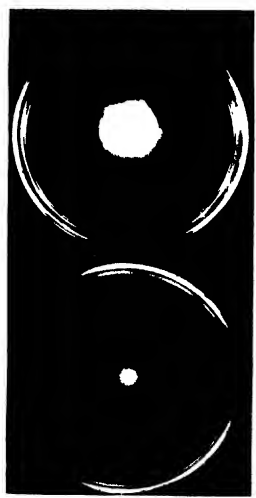


Fig. 2.



Fig. 3.



Fig. 5.

Fig. 6.



Fig. 4.

Fig. 1.



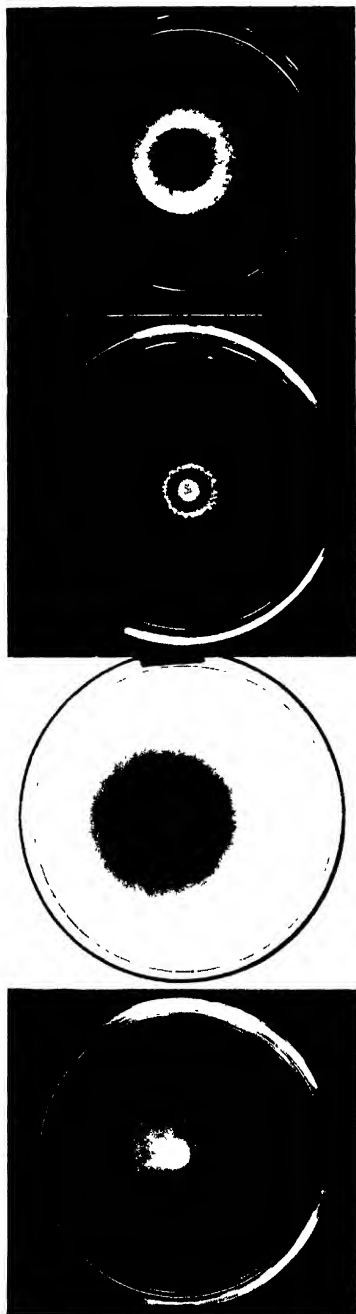


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.



Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.



# THE INTER-RELATION BETWEEN SILICON AND OTHER ELEMENTS IN PLANT NUTRITION

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(With Plate V and 3 Text-figures.)

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## A. INTRODUCTION.

SILICON ranks amongst the elements that are usually found in the ash of plants, and numerous analyses are available to testify to its almost universal occurrence. Wicke (18) found sufficient silicon in beech

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bark to leave a skeleton when properly treated, lesser quantities being detected in other Cupuliferae, in species of *Acer*, and in many members of Urticaceae and Artocarpeae. Hattensaur<sup>(7)</sup> determined in *Molinia caerulea* 28.656 per cent.  $\text{SiO}_2$  in the ash, being 0.646 per cent. of the whole plant, while Ott<sup>(13)</sup> found 70.64 per cent.  $\text{SiO}_2$  in the ash of *Equisetum Telematia* and 41.73 per cent.  $\text{SiO}_2$  in the ash of *Equisetum arvense*. In some cases the silicon content is very heavy, as in *Moquilea* sp. (fam. *Chrysobalaneae*), in which Crüger<sup>(1)</sup> found that the bark contained 30 per cent. ash, of which 96 per cent. was silica. As a general rule silica gradually accumulates with time in the ash, and in young organs the merest traces may occur in plants which, when mature, contain a heavy percentage. The most comprehensive information, however, is given by Wolff<sup>(19)</sup>, who summarises the results of numerous analyses, giving the range and average percentages of  $\text{SiO}_2$  in the ash of many plants. A few of the most typical may usefully be quoted here, to indicate the large amounts of silicon that are normally widely distributed through the vegetable kingdom.

*Percentage of  $\text{SiO}_2$  in ash.*

Plant	Number of analyses	Average	Range
Meadow hay	106	28.73	63.2-10.4
Red clover	113	2.69	20.2- 0.0
Lucerne	12	9.54	27.9- 0.8
Barley (grain)	57	25.91	36.7- 3.7
" (straw)	30	51.00	68.5-32.1
Wheat (grain)	110	1.96	5.9- 0.0
" (straw)	18	67.50	72.5-49.6
Peas	40	0.91	3.0- 0.0
Potato tuber	59	2.04	8.1- 0.0
Sugar beet (root)	149	2.28	12.1- 0.0
" (shoot)	25	10.17	33.5- 0.0

This very general presence of silicon led to the belief that the element was an essential nutrient for many plants, especially cereals, but it has been shown that even such plants which usually contain large quantities of silicon can come to perfect maturity in its absence. The idea also arose that the presence of much silica prevented the lodging of wheat, but as early as 1866 Pierre<sup>(15)</sup> showed this to be a fallacy, as varieties that contain most silica are often the most liable to lodge, as the bulk of the silica is accumulated in the leaves and the least in the nodes. For equal weights leaves contain 7 or 8 times as much silica as the nodes, and

Pierre suggested that if the leaves are removed before the ears ripen, lodging can often be prevented, although much of the silica in the straw is thereby removed. Pfeffer<sup>(14)</sup> supported the theory that the laying of crops after heavy rain is not due to the absence of silica, but rather to the partial etiolation of the basal portions of thickly sown plants.

It is interesting to note that the silicon content of the ash is not necessarily determined by the amount of silica available in the substratum. Richardson<sup>(16)</sup> claims that dune plants grown on almost clear silicate obtain and concentrate in their tissues the same mineral constituents, in approximately the same relative proportion, as the same species grown on ordinary soil, with apparently no extra storage of silica.

The function of silicon in plant nutrition has attracted much attention, both from the theoretical and practical aspects. Fliche<sup>(5)</sup> suggested a possible association between silicon and phosphorus, showing that chalk-avoiding plants like *Calluna vulgaris*, which had about 27 per cent.  $\text{SiO}_2$  in the ash, also had about 10 per cent. phosphoric acid. Hall and Morison<sup>(6)</sup>, after due consideration of the results obtained on certain Rothamsted plots and from special series of water cultures, concluded that the increased and earlier grain formation observed in the presence of silica, is due to an increased assimilation of phosphoric acid within the plant brought about by the silica. They do not cite any evidence that the function of phosphorus is in any degree usurped by silicon, or that phosphoric acid can be replaced by silicates as manurial constituents. Jennings<sup>(8)</sup> found an increase of 17.8 to 29.2 per cent. in dry weight in wheat seedlings grown with 1 per cent. silica added to the nutrient solution, and also an increase in the silica content of the plants<sup>1</sup>. Lemmermann and Wiessmann<sup>(9)</sup> stated that definite increase in yield was induced by silicon in presence of insufficient phosphoric acid, and a less increase if potash was deficient, the best results being obtained with colloidal silicate, this being attributed to an influence exerted on the plants and not on the soil. Later (Lemmermann, Wiessmann and Sammett<sup>(10)</sup>) this view was somewhat modified, and it was further stated that silica does not replace phosphoric acid in the nutrition of the plant, but indirectly increases the amount of phosphoric acid which can be taken up from the soil and the efficiency with which it is utilised. Nanji and Shaw<sup>(12)</sup>, however, controvert this hypothesis, claiming that if phosphoric acid be absent but an abundant supply of silica be available, the latter is able to replace the phosphate

<sup>1</sup> Schollenberger<sup>(17)</sup> also obtained increased growth of various crops by the use of various silicate compounds in soil, both with and without the addition of other fertilisers.



without any detriment to growth; further, that conditions that are favourable for the assimilation of silica may be unfavourable to or even suppress the intake of phosphoric acid.

Densch<sup>(2)</sup> anticipated Lemmermann's results, finding that silica does not replace phosphoric acid, but that soluble silicate induces a stronger growth and greater intake of plant food constituents from the soil. This was further corroborated by Gile and Smith<sup>(3)</sup>, who also found that silica gel greatly benefited the growth of plants receiving rock phosphate, presumably by increasing the quantity of phosphoric acid in solution, though little increase was brought about if acid phosphate were used.

In view of the possibilities of reducing the necessary quantities of phosphate or potassic fertilisers by means of silica compounds suggested by the work of the above investigators and others, experiments have been undertaken at Rothamsted to determine whether the use of silicates, supplementary to other forms of fertilisers, might be an economic proposition.

#### B. RELATION BETWEEN SILICON AND PHOSPHORUS IN PLANT NUTRITION.

With a view to obtaining more exact information as to the possible replacement of phosphorus by silicon in the economy of the plant, the water culture method was employed, enabling silicon to be entirely excluded from the nutritive medium when necessary. The culture bottles were lined with purified paraffin wax to prevent solution of silicate from the glass, and pure analytical salts were utilised for preparing the nutrient solutions. Barley was grown in the presence and absence of phosphate, with or without the addition of soluble sodium silicate. The unit of silicate employed was that providing as much silicon per litre of nutrient solution as is equivalent, atom for atom, to the phosphorus normally supplied in the same amount of solution.

##### I. *Experiment 1, 1924.*

Two types of nutrient solution were employed, of *pH* 6.2 and *pH* 3.7, the only difference being the replacement of the acid potassium phosphate in the latter solution by a mixture of acid and alkaline phosphate in the former to change the *pH* value. Two amounts of silicate were utilised, providing  $\text{Si} \equiv \frac{1}{2}P$  and  $\text{Si} \equiv 1P$ , and as the addition of these caused very considerable fluctuations in *pH* values, a second parallel series was grown in which the correction to the original *pH* was made

by means of standard HCl or NaOH. In this way it was hoped to eliminate variations caused by the varying acidity, and thus to concentrate attention on the effect of the silicate. It was ultimately found that the variations due to fluctuating pH values for the same treatment were very considerable, and for the sake of clearness only that series will here be considered in which the acidity was restored to its original pH value after the addition of silicate. The culture solutions were renewed as required, with five changes in all, the intervals between changes becoming shorter as growth went on. As the bottles were of 600 c.c. capacity, each plant thus had access to 3.0 litres of solution altogether. The pH value of the old solution in each bottle was determined at every change. The composition of the four solutions was as follows:

	With phosphate		Without phosphate	
	A (pH 6.2)	B (pH 3.7)	From A	From B
	gm.	gm.	gm.	gm.
Potassium nitrate	1.0	1.0	1.0	1.0
„ hydrogen phosphate	0.3	0.5	—	—
„ phosphate (alkaline)	0.27	—	—	—
Magnesium sulphate	0.5	0.5	0.5	0.5
Calcium sulphate	0.5	0.5	0.5	0.5
Sodium chloride	0.5	0.5	0.5	0.5
Potassium chloride	—	—	0.4	0.27
Ferric chloride	0.04	0.04	0.04	0.04
Distilled H <sub>2</sub> O to make up 1 litre				

Barley var. Goldthorpe. Graded: .06--.07 gm. Sown: March 3rd, 1924. Put in solution: March 11th, 1924. Harvested: June 11th, 1924.

*a. Phosphorus present.*

Good growth was made in both solutions, though the plants in the more acid one were distinctly more flaccid and less upright, shorter and lighter in weight than the others.

With pH 6.2 solution some increase in height and in green and dry weights occurred with silicate, but the differences were not well marked and probably were of little significance. The proportion of shoot to root, however, was considerably raised by the heavier dose.

With pH 3.7 solution silicate induced a marked increase in height and green weight, but the difference was less marked in the dry weight and shoot/root ratio.

*b. Phosphorus absent.*

Growth throughout was very poor, only one shoot being formed per plant except with the heavier silicate in the pH 6.2 solution, where an

Table I.

*Barley data at harvesting.*

Average of five plants in each set.

Treatment	Height cm.	Number of tillers*	Dry weight			
			Shoot gm.	Root gm.	Total gm.	Shoot Root
A. With phosphate, pH 6.2:						
No silicate	86	9 + 5	12.02	3.03	15.05	3.97
Si≡½P	93	11 + 5	12.62	3.14	15.76	4.03
Si≡1P	91	10 + 4	12.69	2.59	15.28	4.90
B. With phosphate, pH 3.7:						
No silicate	64	11 + 3	9.54	2.27	11.81	4.21
Si≡½P	81	12 + 4	10.18	2.47	12.65	4.13
Si≡1P	81	11 + 3	9.96	2.22	12.18	4.49
A. Without phosphate, pH 6.2:						
No silicate	37	0 + 1	0.302	0.155	0.457	1.95
Si≡½P	54	1 + 0	0.649	0.239	0.888	2.72
Si≡1P	53	1 + 2	1.025	0.401	1.426	2.55
B. Without phosphate, pH 3.7:						
No silicate	27†	0 + 1	0.147	0.085	0.232	1.73
Si≡½P	27†	0 + 1	0.190	0.101	0.291	1.88
Si≡1P	33†	0 + 1	0.257	0.144	0.401	1.78

\* First figure indicates shoots running up to ear; second figure small non-earring tillers.

† Approximately.

*Statistical analysis of total dry weight data.*

	With phosphate	Without phosphate	
		pH 6.2	pH 3.7
Degrees of freedom	40	12	12
Standard deviation of mean = $\sigma_m$	0.6001	0.05652	0.02803
Standard deviation of difference between means = $\sigma(m_1 - m_2)$	0.8467	0.07993	0.03964
Difference required for a probability of .05	1.7100	0.17425	0.08642
„ „ „ .10	1.3920	0.1425	0.0705

average of two tillers appeared. In this solution the addition of silicate caused distinct increase in the average height, green and dry weight of shoot and root, and in the ratio of shoot to root. The extra size of the root in comparison with the shoot was very noticeable in all cases in which phosphorus was absent. The total dry weight was doubled by the lower amount of silicate and tripled by the heavier dose, but even so the plants were very small and in no respect like those receiving phosphorus.

With pH 3.7 the beneficial effect of silicate was much less marked, the lower amount having little or no effect, though the height and

weight were increased somewhat by the heavier amount. In no case did the single shoot show any indication of proceeding to develop an ear.

In this case, therefore, silicate in the presence of phosphate, did little or nothing towards improving growth. In the absence of phosphorus considerable increase in dry weight was affected by the silicate in a solution of a favourable *pH* value, though less advantage was manifested in a more acid solution. These results suggest that under favourable conditions of absorption the silicon had been able to replace the missing phosphorus to some slight extent, and later work was directed towards the elucidation of this point.

(c) *Statistical notes.*

The statistical significance of the observed differences in mean dry weight may be estimated from the variation between replicates as follows. From each group of data within which comparisons of yield are to be made, a single estimate of the standard deviation of parallels is obtained by pooling the variances of all the cultures in the group. Thus there were in the whole experiment 10 cultures (each of 5 plants) receiving full phosphate supply: the standard deviation of parallels for the full phosphate group may thus be based on  $40 = (10 \times (5 - 1))$  degrees of freedom. The standard deviation of the mean of 5 plants,  $\sigma m$ , and of the difference between means of 5 plants,  $\sigma(m_1 - m_2)$ , are calculated from this in the usual way. If the standard deviation had been based on a very large population, a difference between means exceeding twice the standard deviation of the difference between means would occur by chance not more than once in twenty trials (*i.e.* Probability ( $P$ ) = .05) and would be judged significant. When the number of degrees of freedom available for the estimate of the standard deviation is smaller we require more than twice the standard deviation to reach the same level of significance. The values of the factor,  $t$ , by which the standard deviation must be multiplied in order to obtain any given level of significance have been tabulated for small numbers of degrees of freedom by R. A. Fisher(4), and his tables have been used for these calculations. Values of  $\sigma(m_1 - m_2)t$  for  $P = .05$ , *i.e.* values of a difference between means which will not be exceeded by chance more than once in twenty trials, are given, along with values of  $\sigma m$  and of  $\sigma(m_1 - m_2)$  at the bottom of Table I.

In the case of the culture solutions without phosphate the variation between replicates is much greater with the solution of *pH* 6.2 and the standard deviation has been estimated separately for each *pH* group.

The method outlined above for indicating the significance of the yield results will be used for all the experiments to be described in this paper and the values of  $\sigma(m_1 - m_2)t$  for a probability of .05 will be given for each group within which comparisons are to be made.

From the figures at the foot of Table I it is clear that the slight increase in mean dry weight due to the addition of silicate to culture solutions having full phosphate supply is without significance but that the increase due to silicate in the absence of phosphate is fully significant. Moreover the intermediate position occupied by the small dose of silicate ( $\text{Si} \equiv \frac{1}{5}P$ ) leaves no doubt as to the association of increased increments of silicate with increased increment of yield.

With regard to the effect of the plants upon the *pH* value of the solution it was found that in the presence of phosphate the *pH* was rapidly changed to a uniform value of about 6.6, whether the original value was 3.7 or 6.2. In the absence of phosphorus the *pH* reached the same level of 6.6 when it had originally been 6.2, but striking variations occurred with the more acid solution. In this case where no silicate was added the *pH* changed from 5.8 to 6.6 with different plants during the first month, but afterwards the value changed much less and finally remained unaltered. With a light dressing of silicate the initial change was less marked, the value ranging from 4.8 to 5.9, after which it gradually approached the original 3.7, whereas with the heavier silicate the initial change was to 6.2, but less alteration occurred later, the final readings being from 4.8 to 5.6. Normally growing plants tend to stabilise the *pH* value of nutritive solution somewhat on the acid side of neutral, but in the absence of phosphate the normal functioning of the roots did not continue for long, and they became gradually less able to alter the *pH*, though with the heavier silicate this change was less marked.

## II. *Experiment 2, 1925.*

In this second test, attention was concentrated on work with a single solution with and without phosphorus, the *pH* value being modified after the addition of silicate by HCl or NaOH to bring it to approximately 6.2. A certain latitude was allowed owing to the extreme difficulty of making exact adjustment at every change, but the general range was from *pH* 5.9 to 6.3, with occasional slight digression above or below. The amounts of silicate added were  $\text{Si} \equiv 1P, \equiv 2P, \equiv 4P$  and in some cases  $\equiv 8P, \equiv 16P$ , with controls without silicate. The sodium silicate used in this case was the "C soluble silicate" (Brunner Mond) which was found to give the best results in soil cultures in 1924 (see

pp. 65, 66). In addition, a series was tested in which a very little phosphorus equal to the amount in ten barley grains ( $\cdot 00641$  gm.  $P_2O_5$ ) was added to each bottle. This represented  $\cdot 0401$  of the usual quantity of phosphate used in the nutrient solution, *i.e.* 159.9 mg.  $P_2O_5$  per bottle or 266.5 mg. per litre.

Barley var. Spratt Archer. Graded:  $\cdot 04$ – $\cdot 05$  gm. Sown: March 7th, 1925. Put into solution: March 17th, 1925. Harvested, 1st half: June 11th; 2nd half: July 28th, 1925.

Bottles of 600 c.c. capacity were used, and the solutions were the same as the A solutions (sodium chloride being omitted), with and without phosphate, in the 1924 experiment, with the addition of a third containing 6.41 mg.  $P_2O_5$  per bottle. The plants taken off at the first harvest had six changes of solution, and those at the second harvest thirteen changes.

At intervals during the course of the experiment quantitative observations were made on all the plants with a view to obtaining an analysis of the effect of phosphate and of silicate upon the yield in terms of their effect upon the growth processes of the plant. Measurements were made of total height (to the tip of the longest leaf on the main shoot), width of leaves, number of tillers, and, in the later stages of growth, height of the ear. Development curves were thus obtained for certain aspects of growth. A discussion of the results for growth in height will illustrate the kind of information obtained by means of this technique.

(a) *Growth in height and leaf development.*

Fig. 1 shows the total height of certain selected cultures at intervals up to the time of the first harvest. In order to avoid confusion of the curves only six of the cultures are represented, showing the growth at the three levels of phosphate supply (full, little, none) and the effect of sodium silicate at each level<sup>1</sup>. For the cultures without silicate the curves run together until about the 20th day when a sharp divergence begins. As compared with the full phosphate culture that having a little phosphate lags markedly in the middle stages of growth but shows a recovery later, during the period of "shooting." The lag in the no-phosphate culture is still more marked and the recovery both very slight and longer delayed. There is in fact an actual decrease in height in the middle period owing to the shrivelling of the tips of the leaves. The effect of the addition of silicate ( $Si \equiv 8P$ ) at this low level of phos-

<sup>1</sup> The silicate dressing which produced the maximum effect in each case is chosen for representation.

phate nutrition is to delay the marked divergence from the full phosphate culture until nearly the 30th day, but thereafter, although the advantage in height is maintained, the approximation to the full phosphate curve fails. The period of stationary growth is however shortened and the recovery is earlier and more marked than in the absence of silicate.

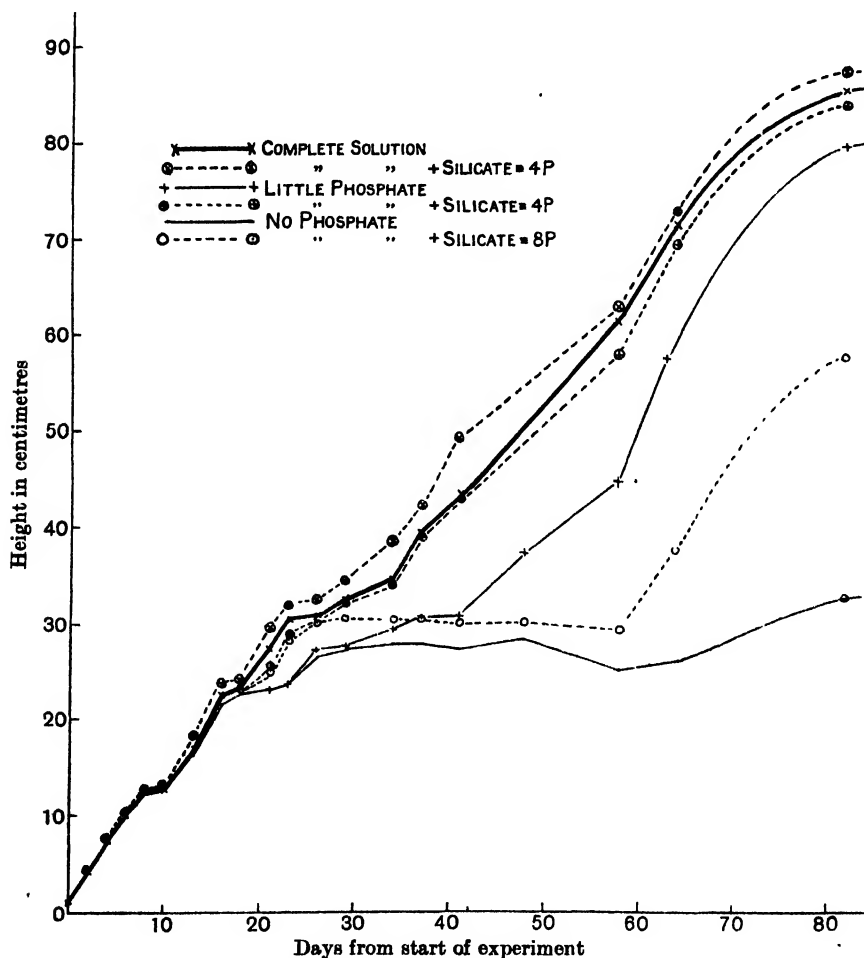


Fig. 1. Total height of main shoot.

The addition of silicate ( $\text{Si} \equiv 4P$ ) to the culture having little phosphate, results in a very close approximation to the behaviour of the full phosphate culture, the lag having almost disappeared. The effect of the addition of silicate to the full phosphate culture is smaller but in

Table II.

*Barley. Data at 1st harvest, 1925.*

Average of five plants in each set.

Average of five plants in each set.							Significant difference between means $\sigma(m_1 - m_2)t$ for a probability of .05	
Treatment	Dry weight gms.			Shoot Root	Dry wt. Ears	% Total plant*	Total dry wt.	Ears as % of total dry wt.
	Shoot	Root	Total					
With phosphate (159.9 mg. $P_2O_5$ per bottle). (Plate V, fig. 2):								
No silicate	10.32	1.39	11.71	7.42	0.521	4.06	2.952	2.402
Si $\equiv$ 1P	10.98	1.69	12.67	6.50	0.533	4.18		
Si $\equiv$ 2P	11.42	1.41	12.83	8.10	1.077	8.22		
Si $\equiv$ 4P	11.33	1.70	13.03	6.66	1.270	9.74		
No phosphate (Plate V, fig. 1):								
No silicate	0.257	0.092	0.349	2.79	0.003	0.89	0.1544	3.510
Si $\equiv$ 1P	0.308	0.093	0.401	3.31	0.014	3.45		
Si $\equiv$ 2P	0.389	0.121	0.510	3.21	0.026	4.71		
Si $\equiv$ 4P	0.557	0.152	0.709	3.66	0.056	7.63		
Si $\equiv$ 8P	0.723	0.242	0.965	2.99	0.121	12.69		
Si $\equiv$ 16P	0.612	0.172	0.784	3.56	0.055	6.45		
Little phosphate (6.41 mg. $P_2O_5$ per bottle):								
No silicate	2.16	0.32	2.48	6.75	0.196	8.87	0.876	4.425
Si $\equiv$ 1P	1.60	0.25	1.85	6.40	0.173	9.49		
Si $\equiv$ 4P	6.14	0.89	7.03	6.90	0.666	9.90		

\* The figures in this column are the means of the values for the five plants in each set.

Table III.

*Barley. Data at 2nd harvest, 1925.*

Survey. Data at 2nd Harvest, 1923.							Significant difference between means $\sigma(m_1 - m_2)t$ for a probability of .05	
Treatment	Dry weight gms.			Shoot Root	Dry wt. Ears	% Ears Total plant*	Total dry wt.	Ears as % of total dry wt.
	Shoot	Root	Total					
With phosphate:								
No silicate	18.30	1.80	20.09	10.17	4.10	17.54	4.320	6.72
Si $\equiv$ 1P	24.27	2.36	26.63	10.28	6.29	23.63		
Si $\equiv$ 2P	21.93	2.37	24.30	9.25	6.13	24.63		
Si $\equiv$ 4P	20.84	1.92	22.76	10.85	6.49	28.54		
No phosphate:								
No silicate	0.284	0.078	0.362	3.64	0.022	5.50	0.1369	7.18
Si $\equiv$ 1P	0.439	0.100	0.539	4.39	0.060	10.92		
Si $\equiv$ 2P	0.558	0.150	0.708	3.72	0.088	12.48		
Si $\equiv$ 4P	0.699	0.197	0.896	3.55	0.153	16.98		
Si $\equiv$ 8P	0.871	0.162	1.033	5.38	0.294	28.30		
Si $\equiv$ 16P	0.586	0.100	0.686	5.86	0.105	13.64		

\* The figures in this column are the means of the values for the five plants in each set.



the same direction, the improvement in growth being greatest in the middle period.

For the first 40 days of growth height measurements were taken at intervals sufficiently close to make possible a calculation of the change in rates of growth with time.

Fig. 2 shows the linear rate of growth in height (centimetres per day) for the two cultures with full phosphate ( $\text{Si} \equiv 0$  and  $\text{Si} \equiv 4P$ ) and the two with no phosphate ( $\text{Si} \equiv 0$  and  $\text{Si} \equiv 8P$ ). The two with "little

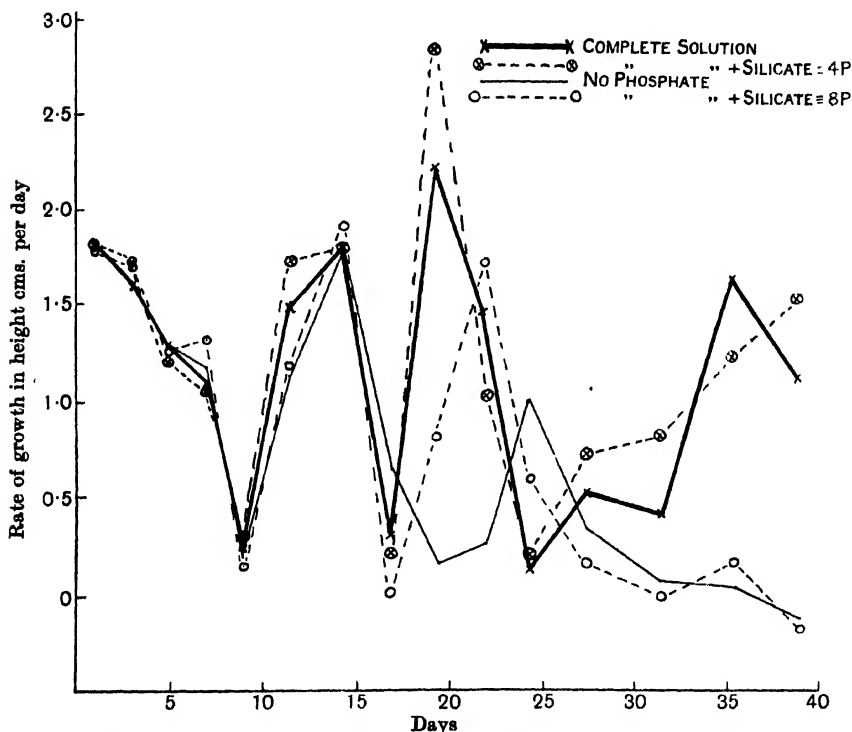


Fig. 2. Rate of growth in height as affected by phosphate and by silicate.

phosphate" are omitted to avoid confusion. Considering first the curve for full phosphate without silicate, the first part of the curve represents the close of the grand period of growth of the first leaf. The second peak of the curve belongs to the second leaf, which now overtakes the first leaf and becomes the longest; the third peak is similarly associated with the elongation of the third leaf. Owing to the facts that after this point younger leaves tend to overlap their predecessors while these are still growing appreciably and that individual plants in the set do not "keep step" quite so well, the grand periods of subsequent leaves become

merged, but the two slight peaks at 27.5 days and at 35.5 days correspond roughly to the growth of the 4th and 5th leaves. The curve for the no-phosphate culture, without silicate, shows, as compared with the normal, a progressive shift to the right in its peaks and a progressive diminution in general level. The effect of sodium silicate at this level of phosphate nutrition is to bring the curve back towards the normal. At the level of full phosphate supply the effect is again a shift to the left, though the difference is very slight.

It is quite clear that, beginning with the second leaf, the effect of phosphate deficiency is to delay the development of the leaves, while the addition of sodium silicate hastens their development. The following table, derived from periodic measurements of the lengths of the last three leaves on the main shoot, shows the time at which successive leaves as they emerged reached the same height as the leaf immediately preceding them and demonstrates this effect of phosphate and of silicate on the rate of emergence of leaves.

Table IV.

*Rate of leaf development.*

Days required for successive leaves to reach same height as leaf immediately preceding them.

	$L_2 - L_1$	$L_3 - L_2$	$L_4 - L_3$	$L_5 - L_4$	$L_6 - L_5$
Full phosphate	10.0	19.5	28.5	33.75	40.0
„ + Si $\equiv$ 4P	9.75	19.25	26.75	32.50	37.75
Little phosphate	12.0	23.0	36.0	43.0	55.0
„ + Si $\equiv$ 4P	11.5	20.5	28.5	35.0	43.0
No phosphate	10.75	23.5	39.0	58.0	67.0
„ + Si $\equiv$ 8P	10.75	20.5	35.5	48.0	58.25

It will be seen that for the stage when the 6th leaf has just become the longest leaf the lag in development for "little phosphate" is 15 days, and for no phosphate 27 days, these periods being reduced by the addition of silicate to 3 days and 18.25 days respectively.

To simplify discussion, data have been given for one concentration of silicate only at each level of phosphate supply: cultures with intermediate concentrations of silicate occupied intermediate positions for each of the aspects of growth considered, with the exception of the no-phosphate culture with 16 units of silicate, which was less advanced than the culture with 8 units and approximated to the culture with 4 units. (The yield of this culture shows a similar behaviour, see Tables II and III.)

The total number of leaves developed on the main shoot averaged ten and no consistent difference in total number as between plants with

and without phosphate could be established. The marked effect of silicate and of phosphate upon the time at which successive leaves emerge is thus accompanied by a similar effect upon the development of the final meristem of the main shoot, that is the ear. Developmental stages tend to be somewhat telescoped in the later stages of growth of the more backward plants, so that the lag in ear development is not as great (measured in days) as the lag in development of the 6th leaf. It is however very well marked and of considerable importance. At any one time a rough measure of the developmental stage reached by different plants may be obtained from the height of the "shoot" (combined leaf sheaths) relative to the total height, or of the height of the ear swelling within the "shoot" relative to the height of the "shoot." Data for the relative height of the ear in the main shoot for all cultures are given in Table V for a date just before the first harvest.

Table V.

*Height of ear relative to total height, June 7th, 82 days from start.*

Full phosphate	Si ≡ 0	77.5 %	} Height of ear is measured to middle of ear swelling.
	Si ≡ 1	85.4	
	Si ≡ 2	87.5	
	Si ≡ 4	91.6	
Little phosphate	Si ≡ 0	75.5	
	Si ≡ 1	73.7	
	Si ≡ 4	86.0	
No phosphate	Si ≡ 0	70.0	
	Si ≡ 1	70.5	
	Si ≡ 2	71.5	
	Si ≡ 4	73.0	
	Si ≡ 8	77.9	
	Si ≡ 16	71.7	

Subsequent measurements of the rate of emergence of the ears from the leaf sheath placed the cultures in exactly the same order. In the case of cultures without phosphate and with only small amounts of silicate the ears did not emerge above the last leaf sheath but protruded laterally through the slit in the sheath.

So far attention has been directed to the time relations of growth, and in particular of leaf development. The size of individual leaves is also affected by silicate and phosphate so that these substances increase the leaf area of the plant both by increasing the rate at which successive leaves emerge and by increasing the area of individual leaves. The following data for the width of the topmost fully unrolled leaf on May 14th, after 58 days' growth will illustrate this effect.

*Leaf width.*

Si≡	Units					
	0 cm.	1 cm.	2 cm.	4 cm.	8 cm.	16 cm.
Full phosphate	1.22	1.35	1.36	1.41	—	—
Little phosphate	0.89	0.86	—	1.16	—	—
No phosphate	0.568	0.640	0.608	0.720	0.720	0.770

*(b) Tillering.*

In view of the marked effect of phosphate and of silicate on the activity of leaf and ear meristems in the main shoot it is not surprising to find the initiation of tillers similarly affected. While, however, in the case of leaves and ears of the main shoot, the total number formed was not affected, in the case of tillering the number of meristems developed is markedly affected and results in considerable differences in total size of plants.

Table VI gives the number of side tillers per plant, for the six cultures previously considered, at intervals up to the time of the first harvest.

Table VI.

*Tillers per plant (average of 5 plants).*

	Days from start												1st harvest. Side tillers with ears
	21	23	26	29	34	37	41	48	58	64	82	86	
Full phosphate:													
Si≡0	0.2	0.7	1.3	2.2	3.3	4.1	5.2	6.2	8.0	9.0	8.9	8.8	7.4
Si≡4	0.8	1.0	1.9	2.3	4.3	5.5	7.2	8.7	11.0	10.5	8.8	8.4	7.6
Little phosphate:													
Si≡0	0.0	0.0	0.0	0.0	0.2	0.2	0.3	0.8	1.8	1.2	1.2	1.25	0.5
Si≡4	0.0	0.6	1.3	1.8	3.2	3.6	4.0	4.5	5.0	5.0	4.4	4.0	3.6
No phosphate:													
Si≡0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Si≡8	0.0	0.0	0.0	0.2	0.2	0.3	0.3	0.2	0.1	0.0	0.0	0.0	0.0

The similarity of the effect of phosphate and of silicate upon rate of tillering and number of tillers developed is evident from the table. What is even more marked is the close association between the level of phosphate supply and the effect of the silicate. Some tillering is indeed induced by the addition of silicate to cultures without phosphate but the tillers die and the effect is very slight. In the presence of little phosphate however, not only does tillering begin eleven days earlier but many more tillers are formed and most of them produce ears. With full phosphate tillering again begins earlier in the presence of silicate and the total number formed is increased. By the time of the first

harvest, the number is reduced, by the death of some, to the same level for both cultures. This number represents in the case of the silicate culture older and more advanced tillers. The average number of side tillers which produced ears was 7.5, a tiller number reached in the case of the silicate culture at 42 days, but reached in the case of the culture without silicate at about 54 days. The ears in the side tillers are in consequence further developed in the silicate cultures, and therefore, although at the first harvest the total number of ears is about the same for both cultures, yet the dry weight of the ears relative to the dry weight of the plant is more than doubled for the silicate culture (see Table II, p. 55). By the time of the second harvest most of the ears have reached maturity and the relative difference in ear weight between cultures with and without silicate is smaller though it remains in the same direction as before (Table II, p. 55).

The significance of the differences in total dry weight between different cultures will be discussed subsequently. It will be convenient however to conclude the discussion of the effect of silicate and of phosphate upon growth by considering the yield of grain.

Table VII.

*2nd harvest.*

	Dry weight of grain gm.	Grains per ear	Fertile %	Mean dry weight of single grain mg.
Full phosphate (no Si)	2.358	7.2	28.8	32.75
"    Si≡ 1P	3.779	10.14	38.3	33.22
"    Si≡ 2P	3.917	9.70	37.3	35.40
"    Si≡ 4P	4.167	12.82	47.1	33.17
No phosphate (no Si)	0.0	0.0	—	—
"    Si≡ 1P	0.0	0.0	—	—
"    Si≡ 2P	0.0062	0.2	—	31.00
"    Si≡ 4P	0.0477	1.4	—	34.07
"    Si≡ 8P	0.1882	10.8	—	15.00
"    Si≡ 16P	0.0288	3.6	—	7.70

Grain weight: significant difference between means for Full phosphate = 1.068; for No phosphate = 0.1732.

The improvement effected by silicate in rate of ear development is here apparent finally in yield per ear, an effect due mainly to an increase in the number of fertile grains, the mean grain weight being, except in the case of the no-phosphate cultures, relatively unaffected.

The emergence of the ears was adversely affected by the absence of phosphorus, as they tended to emerge sideways from the sheath, remaining partially enclosed at the upper end until cut. Gradual improve-

ment occurred in this respect with increasing amounts of silicate, till with  $\text{Si} \equiv 8\text{P}$  normal emergence was attained. Another marked tendency throughout was for the grain to ripen off without filling out properly, this again being improved by silicate.

So far, therefore, as concerns behaviour during growth the effects of phosphate and silicate are strikingly similar in type. The effect of the silicate is however quite definitely a function of the level of phosphate nutrition and, especially as concerns tillering, is relatively very small in the absence of phosphate from the solution.

(c) *Yield.*

The mean dry weights of the plants are given in Tables II and III, and the statistical significance of the differences in total dry weight may be judged from the last two columns which give the value of the difference between means which would not be exceeded by chance more than once in twenty trials. The effect of the silicate at each level of phosphate nutrition is as follows:

*Complete phosphate.*

*1st harvest.*

There is no significant increase in mean dry weight due to silicate either for individual sets or for the average of all the sets with silicate, but the well-marked increase, with increasing silicate, of ear weight as a percentage of the total weight is quite significant.

*2nd harvest.*

Here the culture with  $\text{Si} \equiv 1\text{P}$  is significantly superior in dry weight to that without silicate but it cannot be distinguished from the other silicate cultures. The average of the silicate cultures is significantly greater than the no-silicate culture. Yield of grain moreover shows a significant increase with increasing silicate (see Table VII).

*Little phosphate.*

The yield for  $\text{Si} \equiv 1\text{P}$  is undoubtedly anomalous in being lower than for  $\text{Si} \equiv 0\text{P}$ , but the two yields do not differ by a significant amount and no importance can be attached to the apparent anomaly. The striking increase with  $\text{Si} \equiv 4\text{P}$  is quite significant.

*No phosphate.*

For both harvests. In the series of increasing silicate concentrations the yields increase up to  $\text{Si} \equiv 8\text{P}$  and then fall, and with the exception of  $\text{Si} \equiv 1\text{P}$  in the 1st harvest each step in the silicate series brings about a significant difference in yield. The depression in yield with  $\text{Si} \equiv 16\text{P}$  is quite significant.

## 62 *Silicon and other Elements in Plant Nutrition*

It will be seen that the actual increments in yield due to addition of the same amount of silicate, *e.g.*  $\text{Si} \equiv 4P$ , are very small in the absence of phosphate (+ 360 and + 534), much greater in the presence of a little phosphate (+ 4551) and still appreciable though less definitely significant in the presence of full phosphate (+ 132 and + 267). There is obviously no question of the *replacement* of phosphorus by silicon, but clearly the use that can be made of the phosphorus presented to the plant is affected by the addition of silicate.

### (d) *Uptake of $P_2O_5$ , of $SiO_2$ and of ash.*

The  $P_2O_5$  and ash contents of the water culture plants from the first harvest are given in Table VIII. On account of the small weight of material available from the no-phosphate series the sets were bulked in pairs so as to have 3 samples in the series of increasing silicate concentrations.

*Ash content.* The ash content increases in every case with increasing silicate concentration, the increase being most marked in the roots. The large increase here may be partly due to silica adhering to the roots though great care was taken in washing them. The increase in ash content does not however contribute very greatly to the increase in dry weight of plants with addition of silicate as is shown by the figures for the ash-free dry weight.

*Silica.* The silica content of the roots was not determined owing to the doubt concerning silicate adhering to the root surface. In the shoots the  $SiO_2$  content increases more than 10 times with the addition of  $\text{Si} \equiv 4P$ , but again contributes very little to the increase in dry weight.

*$P_2O_5$  content.* It will be seen that in spite of the fact that A.R. chemicals were used for the culture solutions, yet in the no-phosphate set the plants contain more  $P_2O_5$  than was contained in the grain. The amounts concerned are very small and very slight impurity in the A.R. chemicals would account for the results. A test for phosphorus in the sodium silicate revealed no trace of that element and moreover evidence from the  $P_2O_5$  content of the plants suggests that the effect of the addition of silicate upon the plants cannot be due to traces of  $P_2O_5$  in the silicate. For, in the case of the little phosphate culture the addition of  $\text{Si} \equiv 4P$  results in an increased  $P_2O_5$  uptake of 8.865 mg. while with the no-phosphate culture the increased uptake of  $P_2O_5$  for  $\text{Si} \equiv 3P$  ( $\text{Si} \equiv 2P$  and  $\equiv 4P$  bulked) over  $\text{Si} \equiv \frac{1}{2}P$  ( $\text{Si} \equiv 0P$  and  $\equiv 1P$  bulked) is only 0.239 mg. and that for  $\text{Si} \equiv 12P$  ( $\text{Si} \equiv 8P$  and  $\equiv 16P$  bulked) is only 1.078 mg. If the  $P_2O_5$  were being supplied with the silicate the increase

in  $P_2O_5$  uptake with addition of silicate should be greater with the cultures originally containing the smaller amount of phosphate.

With the no-phosphate solution therefore one is not working with zero concentration of phosphate, but with a very low concentration approaching zero<sup>1</sup>. In the solutions deficient in phosphate the effect of the addition of silicate is a progressive increase in uptake of  $P_2O_5$ . With the full phosphate solution however, the  $P_2O_5$  content is decreased by the addition of silicate. The percentage of  $P_2O_5$  both in dry weight and in ash content decreases with the addition of silicate. Calculated on ash free dry weight this decrease is smaller but still evident, i.e. the increase in ash free dry weight per plant due to the addition of silicate is greater than the increase in  $P_2O_5$  per plant and from the point of view of dry weight production the  $P_2O_5$  in the plant must be regarded as increasing in efficiency as silicate is added. The same feature, an increase in ash free dry weight greater than the increase in  $P_2O_5$  content, is however found in the series of increasing  $P_2O_5$  supply, the plants in the full phosphate solution having the lowest  $P_2O_5$  percentage. In this respect the addition of silicate behaves like an increase in phosphate supply and the effect might be regarded as due to an increased availability to the plant of the phosphate already present in the solution. That there is an effect of this kind seems probable from other work, but the case of the full phosphate solution where, although the dry weight of ears is markedly increased by silicate yet the  $P_2O_5$  content is reduced, suggests that the presence of silica does increase also the efficiency of the  $P_2O_5$  within the plant.

Only a study of the  $P_2O_5$  distribution in the plant and the changes in this distribution with time can clear up this question, but it may be suggested that the silica might act within the plant by unlocking phosphate from relatively quiescent parts of the plant and enabling it to be transferred to regions where assimilation and growth are active.

<sup>1</sup> An approximate estimate of the  $P_2O_5$  concentration in the no-phosphate solution may be obtained from the three points on the curve relating yield to  $P_2O_5$  supplied (otherwise than as impurity). The  $P_2O_5$  supplied in mg. per bottle is 0, 6.41, 159.9, the yields are .349, 2.48, 11.41. Solving the following equation for yield

$$\text{yield} = \frac{1}{k_1 + \frac{k_2}{P+p}},$$

where  $P$  is mg.  $P_2O_5$  supplied and  $p$  is mg.  $P_2O_5$  present as impurity (and in the seed) we have  $k_1 = 0.0703$ ,  $k_2 = 2.4215$ ,  $p = 0.8664$ . The total weight of nutrient salts per bottle in the no-phosphate solution was 1.464 gm. so that an impurity of less than .059 per cent.

$\left( = \frac{.8664 \times 10^3}{1.464 \times 10^3} \right)$  would account for the results.



Table VIII.

Water cultures, 1924 and 1925.

 $P_2O_5$  content, Ash content, and Silica content of plants.

	1925										1924		
	Full phosphate			Little phosphate			No phosphate				No phosphate		
	Si≡0	Si≡4P		Si≡0	Si≡1P	Si≡4P	Si≡0, 1P	Si≡2, 4P	Si≡8, 16P		Si≡0	Si≡½P	Si≡1P
$P_2O_5$ % of dry matter:													
Roots ...	0.657	0.482	...	0.484	0.456	0.388	0.386	0.404	0.378		—	—	—
Shoots ...	0.137	0.120	...	0.213	0.181	0.187	0.197	0.127	0.181		0.161	0.214	0.181
Whole plant ...	0.198	0.167	...	0.248	0.218	0.214	0.244	0.189	0.228		—	—	—
$P_2O_5$ content, mg. per plant:													
Roots ...	9.11	8.19	...	1.535	1.118	3.535	0.357	0.551	0.783		—	—	—
Shoots ...	14.16	13.60	...	4.600	2.905	11.460	0.556	0.601	1.208		0.735	1.900	2.580
Whole plant ...	23.27	21.79	...	6.135	4.023	14.995	0.913	1.152	1.991		—	—	—
Ash % of dry matter:													
Roots ...	19.10	36.20	...	6.740	6.360	16.780	10.20	18.80	30.00		—	—	—
Shoots ...	14.45	15.88	...	8.730	10.750	12.260	14.32	14.88	16.52		16.65	12.08	15.45
Whole plant ...	15.00	18.50	...	8.480	10.190	12.810	13.30	15.75	19.70		—	—	—
Ash free dry wt. gms.:													
Whole plant ...	9.972	10.632	...	2.265	1.670	6.120	0.3252	0.5138	0.7020	Shoots only	0.381	0.781	1.204
$P_2O_5$ % on ash free dry wt.	0.2335	0.2050	...	0.2708	0.2410	0.2450	0.2805	0.2242	0.2840		0.193	0.243	0.214
$P_2O_5$ % of ash wt.	1.323	0.902	...	2.93	2.14	1.662	1.830	1.200	1.158		0.966	1.72	1.170
$SiO_2$ % of dry matter:													
Shoots only ...	0.211	2.500	...	0.151	—	2.350	—	—	3.100		—	—	—
$SiO_2$ % of ash:													
Shoots only ...	1.46	15.75	...	1.73	—	19.20	—	—	18.75		—	—	—

Grain 1924.  $P_2O_5$  % on fresh weight (approx.) .762 %. Average weight of grain .065 gm.  $P_2O_5$  per grain = .4953 mg.Grain 1925.  $P_2O_5$  % on fresh weight, 0.854 %. Average weight of grain .045 gm.  $P_2O_5$  per grain = .3844 mg.

## C. FERTILISING VALUE OF SODIUM SILICATE IN SOIL CULTURES.

With a view to determining whether sodium silicate might be used instead of or in partial replacement of other artificial fertilisers, pot culture experiments were carried out in 1924 and 1925 with various combinations of silicates and manures. In 1924 two soluble silicates, C and M, and one insoluble glass silicate, M, were tested against controls without silicate for each manurial combination. The silicate was applied in quantities bearing the same proportion to the manures used as was the case in field experiments on the farm and provided silicon equivalent to five times the phosphorus in the 5 gm. superphosphate applied per pot. Nitrogen was supplied as sulphate of ammonia or dried blood, phosphorus as superphosphate or gafsa phosphate, and a test with dung was included. Rothamsted heavy loam was used, with the admixture of 10 per cent. sand to lighten it somewhat, and barley and mustard were grown at different seasons.

## I. Series I.

## (a) Barley. Goldthorpe.

Sown: Feb. 15th. 1924. Harvested: July 23rd, 1924.

The influence of silicate was not very marked during growth, though certain differences were shown by the dry weights.

Table IX.

*Dry weights of barley.*

Average of four pots of three plants in each set.									
Manures	No silicate		C soluble silicate		M soluble silicate		M glass silicate		Significant difference in dry wt. (total shoot) $\sigma(m_1 - m_2) t$ for a probability of .05
	Ears gm.	Total Shoot gm.	Ears gm.	Total Shoot gm.	Ears gm.	Total Shoot gm.	Ears gm.	Total Shoot gm.	
1. S/A, Super, $K_2SO_4$	30.53	60.48	35.80	<b>69.8</b>	35.2	<b>68.5</b>	31.8	62.0	5.325
2. Blood, Super and $K_2SO_4$	32.81	63.16	33.7	66.6	36.5	<b>70.4</b>	33.2	65.9	4.840
3. Super, $K_2SO_4$	20.74	45.24	24.8	<b>51.0</b>	21.9	48.2	21.2	48.0	4.170
4. S/A, $K_2SO_4$	30.11	59.81	32.5	<b>63.8</b>	31.9	<b>63.2</b>	31.5	<b>62.35</b>	2.203
5. S/A, Super	32.48	63.60	34.0	65.9	36.5	68.5	32.9	63.1	6.050
6. S/A, Gafsa and $K_2SO_4$	28.17	58.67	32.6	63.9	32.4	64.4	30.2	59.5	7.120
7. Dung	30.08	62.96	31.1	63.6	28.6	59.8	26.16	<b>56.62</b>	4.280
8. No manure	19.81	43.37	22.0	<b>48.9</b>	21.77	<b>48.02</b>	19.16	42.18	2.050
	Mean 57.15		61.685		61.371		57.465		1.610
Average for whole experiment									4.550

The level of significance for each type of basal manuring is given in the last column of Table IX and in the columns for the three silicate manures, yields which differ significantly from the corresponding control are in heavy type. In the first column, for yield in absence of silicate, yields differing significantly from the yield for complete manure (No. 1) are in italics. The only basal manurings differing in yield from the complete manure are "no nitrogen" (No. 3) and "no manure" (No. 8), other variations in manuring being without effect. In the case of the "no manure" set the yield is increased by both the soluble silicates, the difference between no manure and complete being reduced by about 30 per cent. Much the same increase with silicate occurs in the no-nitrogen set, though here owing to the greater variation of parallel pots, only the increase due to the C soluble silicate is of undoubted significance.

For the other types of basal manuring we have a significant increase with all three silicates for the no-phosphate series (No. 4) and an increase with the soluble silicates for the no-potash series (No. 5): the latter increase is not however significant. The greatest increase is that given by the two soluble silicates in the presence of complete manure and is undoubtedly significant. On the whole the increases in yield are as marked with the more complete manures as with the deficient manures (with the exception of dung where the M glass silicate produces a significant depression). Averaging all the results the two soluble silicates produce a significant increase in yield of 7.8 per cent. while the M glass silicate is without effect. The proportion of dry matter in green was not affected by the soluble silicates but had a tendency to be higher with M glass silicate, especially in the absence of potash.

(b) *Mustard.*

Sown: Aug. 13th, 1924. Harvested: Nov. 13th, 1924.

Table X.

Average of four pots of three plants in each set.

	No silicate	C soluble silicate	M soluble silicate	M glass silicate	Significant difference in dry wt. $\sigma(m_1 - m_2) / t$ for a proba- bility of .05
	Dry wt. gm.	Dry wt. gm.	Dry wt. gm.	Dry wt. gm.	
1. S/A, Super, $K_2SO_4$	23.98	22.48	24.28	22.24	2.99
2. Blood, Super, and $K_2SO_4$	19.53	<b>23.13</b>	20.08	20.58	2.20
3. Super, $K_2SO_4$	5.43	5.49	5.08	4.98	0.68
4. S/A, $K_2SO_4$	14.88	20.90	12.95	15.45	6.28
5. S/A, Super	20.05	23.15	22.58	22.03	3.42
6. S/A, Gafsa, and $K_2SO_4$	20.32	20.83	17.95	18.39	4.21
7. Dung	6.03	5.65	6.10	6.25	1.154
8. No manure	5.05	<b>7.28</b>	5.45	5.63	1.145
Mean	14.41	16.12	14.31	14.44	1.081
Average for whole experiment					3.065

As before, yields with silicate differing significantly from the yields of the corresponding control (Table X) are in heavy type, and yields without silicate differing significantly from the complete manure (No. 1) are in italics. Those under which there is a broken line show differences (from their control) for which P (Probability) lies between .05 and .10.

In marked contrast to the results with barley on the same soil all manurings other than the complete (No. 1) give significantly lower yields, and it is only upon these deficient manurings and not at all upon the complete manuring that silicate has any effect, and then only the C soluble form is effective. The manurings which show a response to this silicate are "no manure" (No. 8), where the increase is quite small but significant, "no superphosphate" (No. 4), where the increase is considerable and probably though not certainly significant, no potash (No. 5), where the increase is small and of doubtful significance, and dried blood (No. 2), where the increase is small but significant. So far as they go the results with mustard are consistent with the theory of a replacement of phosphate and possibly of potash manures by silicate.

Considering the results from both crops it is evident that the soluble silicates were the more active, especially the C soluble form, and that they tended to cause increase in dry weight with deficient mineral manuring, especially phosphorus, and occasionally benefit occurred even with complete manuring.

## II. *Series II.*

As silicates had proved capable of partially replacing phosphorus, and possibly potash, in artificial fertilisers, an attempt was made in 1925 to determine whether such replacement could be made economically, and the proportion of silicate required for the purpose. In order to give the sodium silicate full play special Cheshire soil was obtained, deficient in phosphate, potash and lime. The lime deficiency was corrected by the addition of sufficient calcium carbonate to bring the pH value of the soil to about 7.0. The unit of silicate applied was that containing as much silicon as is equivalent to the phosphorus in 5 gm. 15.93 per cent. superphosphate. This unit was adopted for convenience, as the complete fertiliser applied to each pot containing 21 lbs. soil consisted of

5 gm....	...	...	...	15.93 per cent. Super.
2 gm....	...	...	...	K <sub>2</sub> SO <sub>4</sub>
2.5 gm.	...	...	...	S/A

The range of silicate tested was

$$\text{Si} \equiv 0P, \equiv 1P, \equiv 2P, \equiv 4P, \equiv 8P, \equiv 16P.$$

The actual quantities of the C soluble silicate applied per pot were therefore

0P, none;  $\equiv$  1P, 1.324 gm.;  $\equiv$  2P, 2.648 gm.;  $\equiv$  4P, 5.296 gm.;  
 $\equiv$  8P, 10.592 gm.;  $\equiv$  16P, 21.184 gm.

The addition of so much silicate had a considerable effect on the pH value of the soil, and had this not been compensated for, it would have been impossible to estimate how far the results were influenced by varying alkalinity. Sufficient hydrochloric acid was therefore added to neutralise the silicate in each case. To ensure adequate distribution the silicate for each pot was mixed with a small quantity of dried soil, the acid (made up to a standard quantity throughout to avoid affecting the water content) was pipetted over and the whole thoroughly incorporated. This was then sprinkled over the bulk of the soil, with the requisite manure, and all well mixed together. In this way it is hoped that the formation of acid and alkaline "pockets" in the pots was avoided, and a uniform distribution of silicate and fertilisers obtained.

(a) *Barley. Spratt Archer.*

Seed graded: .04-.05 gm. Seed sown: March 13th, 1925. Harvested: July 31st, 1925.

The manurial scheme was:

1. No manure.
2. 2.5 gm. S/A, 2 gm.  $K_2SO_4$ .
3. 2.5 gm. S/A, 2 gm.  $K_2SO_4$ , .0241 gm. Super.
4. 2.5 gm. S/A         —       5 gm. Super.
5. 2.5 gm. S/A, 2 gm.  $K_2SO_4$ , 5 gm. Super.

(a) *Growth and maturity.* During the growth the most noticeable feature was the general, though not universal, improvement with increasing doses of silicate. This was particularly striking, and somewhat unexpected, with the unmanured plants, which reached a development with heavy dressings of silicate approximating to that of plants receiving nitrogen and potash in addition. At the time of harvesting all plants receiving phosphate, with or without potash, were nearly dead ripe, irrespective of the silicate dressing. With N and K, but no P, maturity was much less advanced, but improved with increasing silicate, though even with Si  $\equiv$  16P much green colour was still observable in leaves and ears. With no manure at all, *i.e.* in absence of N and K as well as P, all plants were a little riper than those receiving N and K, and again ripeness increased with silicate.

Silicate, therefore, acted in the same sense as phosphate, in hastening maturity, although even with the heaviest dressing it was less effective in that respect than a single dose of 5 gm. superphosphate.

( $\beta$ ) *Discussion of dry weights.*

Table XI.

*Barley. Total plant. Dry weight.*

Average of four pots of three plants in each set.

	Si	$\equiv 0P$ gm.	$\equiv 1P$ gm.	$\equiv 2P$ gm.	$\equiv 4P$ gm.	$\equiv 8P$ gm.	$\equiv 16P$ gm.	Significant difference $\sigma(m_1 - m_2) t$ for a proba- bility of .05
1. No manure		17.341	22.699	21.179	24.653	29.570	30.609	5.55
2. N + K (no P)		30.695	30.249	32.932	29.719	31.437	32.574	3.07
3. N + K + little P		29.569	29.019	31.092	31.300	30.604	33.005	4.17
4. N + P (no K)		34.854	41.961	45.838	47.065	47.240	51.807	5.51
5. N + K + P		48.490	49.078	50.088	49.118	50.139	54.776	4.27
Average for whole experiment								4.435

Table XII.

*Barley. Ears. Dry weight.*

	Si	$\equiv 0P$ gm.	$\equiv 1P$ gm.	$\equiv 2P$ gm.	$\equiv 4P$ gm.	$\equiv 8P$ gm.	$\equiv 16P$ gm.
1. No manure		7.459	10.716	9.342	11.462	14.552	14.103
2. N + K (no P)		12.366	12.271	13.478	12.750	13.741	14.303
3. N + K + little P		12.149	11.065	13.502	13.258	13.573	14.578
4. N + P (no K)		16.984	21.104	23.972	23.935	23.093	22.994
5. N + K + P		23.390	24.268	24.092	22.730	23.175	22.021

Table XIII.

*Barley. Grain. Dry weight.*

	Si	$\equiv 0P$ gm.	$\equiv 1P$ gm.	$\equiv 2P$ gm.	$\equiv 4P$ gm.	$\equiv 8P$ gm.	$\equiv 16P$ gm.	Significant difference $\sigma(m_1 - m_2) t$ for a proba- bility of .05
1. No manure		5.721	9.123	7.449	9.110	11.668	11.296	2.64
2. N + K (no P)		9.996	9.467	10.469	10.309	10.648	11.646	1.76
3. N + K + little P		9.583	8.462	10.959	10.064	10.874	11.667	1.89
4. N + P (no K)		13.555	17.093	20.151	19.275	18.744	18.252	3.315
5. N + K + P		18.904	20.001	19.474	18.780	19.000	17.731	3.095
Average for whole experiment								2.498

With barley, on the light Cheshire soil, deficient in available phosphorus and potash, silicate failed to bring about any appreciable improvement when phosphorus was omitted from the manure, and it was therefore not replacing the phosphorus *per se* nor unlocking stores of

phosphorus in the soil and rendering them available as plant food (Table XI). The large increase with silicate when *potash* was omitted suggests that the sodium silicate was either replacing or unlocking potash (Plate V, fig. 3). The presence of the base sodium as well as silicon renders it impossible to determine which of the elements was the active agent, or whether the compound as such was effective. It is conceivable that the sodium might have functioned as potassium in the economy of the plant or that it might have replaced potassium in soil compounds, thus freeing the latter element and rendering it available for use. On the other hand, the silicon may have played some part in plant nutrition or soil conditions for which no explanation can be offered. The heavy increase with silicate in the absence of any other fertiliser is noteworthy, as the  $\text{Si} \equiv 16\text{P}$  was as effective as a dressing of N and K, and nearly as much so as  $\text{N} + \text{P}$ , and it is difficult to formulate a reason for so marked a beneficial action. Unfortunately owing to limitations of space no tests were made with K and P in the absence of nitrogen, so no information is available as to the need of the soil for nitrogenous manuring.

In view of the marked effect, noted in the water culture experiments, of silicate upon ear formation and on the percentage of fertile florets a careful examination of all the ears of the plants in this experiment was made (Tables XII and XIII). It will be convenient to summarise the results in two tables showing (1) the average effect of silicate irrespective of other manuring (Table XIV), (2) the average effect of other manuring irrespective of silicate (Table XV).

Table XIV.

A. *Effect of silicate dressing.*

	Si	$\equiv 0\text{P}$	$\equiv 1\text{P}$	$\equiv 2\text{P}$	$\equiv 4\text{P}$	$\equiv 8\text{P}$	$\equiv 16\text{P}$
Ears per pot		14.95	15.77	17.39	16.95	17.50	18.05
Florets per ear		29.46	29.66	27.50	28.46	28.88	29.12
Fertile florets (%)		82.24	81.82	81.74	77.86	77.08	72.12
Average grain wt., mg.		30.43	32.80	33.89	35.41	36.33	37.62

Table XV.

B. *Effect of basal manuring.*

	No manure	No P	Little P	No K	Complete
Ears per pot	11.93	14.24	13.97	21.78	21.92
Florets per ear	28.53	28.14	28.23	29.62	29.80
Fertile florets (%)	78.97	81.02	82.58	76.73	74.58
Average grain wt., mg.	33.16	31.78	31.60	36.40	39.15

The major effect of silicate dressing and of variation in basal manuring is on the number of ears per pot; upon the average number of florets per ear neither has much effect but both again affect the percentage of fertile florets, which decreases with increased silicate and with the addition of superphosphate and potash. This decrease runs parallel with the increase in the number of ears and reduces the grain yield below what would be expected from the increase in ear number. A further compensation, however, is found in the average weight of grain, which is affected by both silicate and the other manures in exactly the opposite direction to percentage fertility.

Taking the two basal manures which showed the greatest response to silicate dressings, no manure (Table XVI) and no potash, the major part of the increase in grain yield with increasing silicate can be ascribed to an increased number of ears per plant, while the effects upon percentage of fertile florets and upon average grain weight are smaller and tend to counterbalance one another.

Table XVI.

*Effect of silicate in presence of no manure.*

Si	≡0P	≡1P	≡2P	≡4P	≡8P	≡16P
No. of ears	9.25	11.3	11.7	12.25	12.75	14.25
Florets fertile (%)	82.5	76.0	86.0	86.3	74.7	80.6
Average grain wt., mg.	27.9	33.4	32.1	34.3	34.8	36.3
Grain yield, gm.	5.72	9.12	7.45	9.11	11.67	11.30

*Effect of silicate in presence of no potash.*

No. of ears	17.5	20.75	21.50	23.25	22.75	25.00
Florets fertile (%)	79.9	82.9	85.00	74.3	74.2	64.1
Average grain wt., mg.	31.4	33.9	36.9	37.7	38.1	40.4
Grain yield, gm.	13.55	17.09	20.15	19.28	18.74	18.25

Thus the results for pot cultures differ considerably from those for water cultures, where the main effect of silicate upon yield of grain lay in an increase in number of fertile grains and not at all in increased number of ears or increased grain weight. Comparison between the two sets of conditions is however difficult, and as regards percentage of fertile grain it is clear that the conditions were very different, since the percentage fertility ranges from 28 to 47 per cent. in the case of the water cultures and from 64 to 88 per cent. in the pot cultures. The very small effect of silicate in the absence of superphosphate, an effect moreover revealed also by the analysis of the grain yields, is also surprising.





It has already been shown that with barley the omission of either potash or phosphorus from a complete manure (without silicate) had a very similar effect in depressing the dry weight, the absence of P being a little more harmful. With mustard, on the same light Cheshire soil, the omission of phosphorus vitiated the beneficial action of the added N and K, so that the dry weight rose very little above that when no manure at all was added. With increase in the phosphate supplied a gradual improvement in dry weight set in. The addition of silicate brought about a greater increase of dry weight with deficient phosphorus than with deficient potash, suggesting that in this case the silicate is either releasing some of the phosphorus locked in the soil, or that the silicon is partially replacing phosphate (Plate V, fig. 4). With mustard, as with barley, the considerable increase with silicate in the absence of any other fertiliser is noteworthy, the addition of  $\text{Si} \equiv 16\text{P}$  being as effective as a dressing of N and K with some amount of superphosphate between 0.2 and 1.0 gm. per pot.

Comparison may be made of the effect of silicate on the heavy unlimed Rothamsted soil and the light limed Cheshire soil in which the alkalinity induced by the silicate was rectified by the addition of hydrochloric acid. With *barley* on both soils silicate was beneficial in differing degrees in the absence of manure and also with complete fertilisers. In the absence of potash, however, silicate was less beneficial on the Rothamsted soil than on the Cheshire type, whereas in the absence of phosphorus it did cause a slight improvement, the difference in the potash and phosphorus needs of the two soils being probably sufficient to explain this variation. With *mustard* the results were similar on both soils, except that as the Rothamsted soil was initially less deficient in phosphorus the extreme depression in its absence from a mixed manure was less evident.

( $\gamma$ ) *Statistical consideration of results.* The general similarity of the effect of silicate and of phosphate upon yield is clear from the figures in the table. The series of increasing doses of superphosphate was introduced in the hope of enabling one to characterise more precisely the type of interaction between silicate and phosphate that is involved. From the point of view of the yield-factor relationship<sup>(11)</sup> two simple alternatives present themselves: (1) the addition of unit amount of silicate is equivalent to the addition of a fixed amount of superphosphate, (2) the addition of unit amount of silicate is equivalent to increasing the amount of superphosphate already present in a fixed ratio. In the first case we might suppose that unit amount of silicate could replace so much

phosphorus within the plant or could set free from the soil so much phosphate. In the second case we might suppose that the efficiency of the phosphorus within the plant was increased in a fixed ratio by the presence of so much silicate or that the fraction of the total superphosphate supply which is available to the plant is similarly increased in a fixed ratio.

On the first supposition the silicate effect might be described in terms of "equivalent increments of superphosphate," on the second it would be described in terms of "relative efficiency of superphosphate." The difference between the two alternatives may be appreciated by

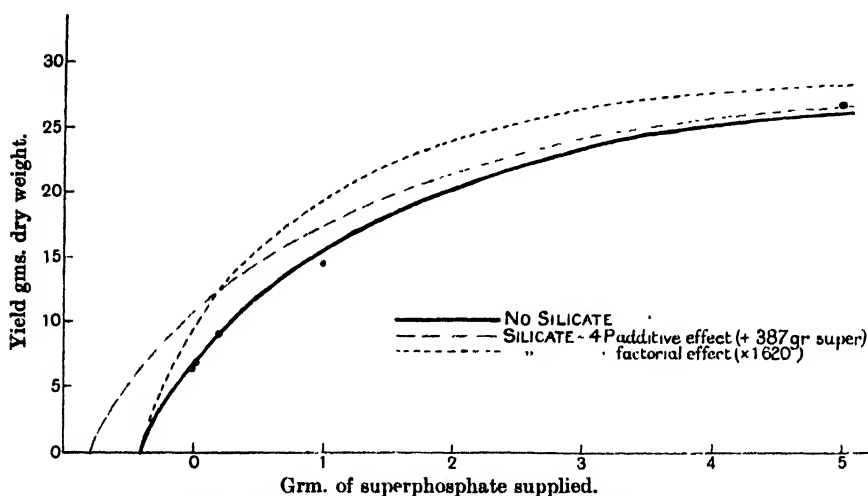


Fig. 3. Mustard. Yield—superphosphate relationship.

reference to Fig. 3 where the continuous line represents the simple yield-superphosphate relation for this experiment, and the dotted lines show how the uniform addition of a fixed dose of silicate would on either of the above suppositions affect the yield<sup>1</sup>. With the exact form of the yield-superphosphate relation we are not for the moment concerned, other than to note that it is of the "diminishing returns" type. The important point is that if the silicate effect is represented as equivalent to an increment of superphosphate then the yield curve is simply shifted to the left, with the consequence that the increment of yield due to silicate is maximal at zero superphosphate supply and rapidly diminishes as superphosphate increases. If it is, however, an effect on the

<sup>1</sup> The two curves for the effect of silicate are those for the effect of four times the unit dose of silicate in this experiment and are based on Table XIX A and B.

efficiency of the superphosphate already present, then the origin of the yield curve remains fixed but the scale of the horizontal axis contracts, so that the increment of yield due to silicate is zero at zero superphosphate, increases at first with increasing superphosphate, and then diminishes again to zero.

We saw earlier (p. 54) that the water culture results with barley were of the second type, the increase in yield with silicate being quite small with "no phosphate," very considerable with little phosphate, and small with full phosphate. Again the evidence from the  $P_2O_5$  content of the water culture plants suggested an effect of silicate upon the efficiency of phosphorus within the plant, which would be consistent with this type of yield relationship.

The yield data for the mustard may now be examined. The first essential is an equation for the yield-superphosphate relation. Several types of equation, including the well-known equation of Mitscherlich are available. There are reasons (11) however for preferring an equation of

the form  $Y$  (Yield) =  $\frac{1}{k_1 + \frac{k_2}{p + P}}$ , where  $k_1$  and  $k_2$  are constants,  $P$  is the

amount of superphosphate added to the soil, and  $p$  is the equivalent amount of superphosphate already present in the soil. The effect of the addition of silicate is to be represented either as an addition,  $P'$ , to the function  $(p + P)$ , or as the multiplication of the function  $(p + P)$  by a definite factor  $E$ . The procedure adopted has been to calculate the constants for the 5 yields for increasing superphosphate without silicate and to apply this equation to the yields with silicate. Selection of all possible groups of 3 observations from the 5 observed yields gave 10 sets of simultaneous equations from each of which by eliminating  $k_1$  and  $k_2$  a value for  $p$  was obtained. These 10 values were weighted by the mean yield for each of the 10 sets of observations and the weighted mean of these values taken to form a new set of equations linear in form from which Gauss' least square method  $k_1$  and  $k_2$  are found. The calculated

equation is  $\text{Yield} = \frac{1}{\cdot 02922 + \frac{\cdot 0503}{\cdot 406 + P}}$ , the calculated yields being 6.52,

6.84, 8.90, 15.38, 25.99 and the observed yields 6.523, 7.023, 8.970, 14.590, 27.670. The sum of squares of deviations of calculated from observed values is 3.4845 and the variance is half this—1.74225—since after fitting 3 constants only 2 degrees of freedom are left. For the whole experiment the variance of parallels is 2.2421 (126 degrees of

freedom), *i.e.* greater than the variance of deviations from this fitted equation: the variance of parallels for the 5 sets without silicate involved in the fit is however much smaller 1.2657 (15 degrees of freedom). The divergence between the variance of parallels and that of deviations is not however significant.

The calculated equation may accordingly be used as a basis for the analysis of the silicate effect. For the moment the series of silicate dressings with full superphosphate manuring will be omitted from consideration since the variation due to silicate dressing within that set is very little greater than the random variation of parallels. For the other levels of superphosphate manuring the value of  $P + P'$ , *i.e.* the equivalent amount of superphosphate required to give the observed yield for each combination of silicate and superphosphate, may be calculated. (In the equation,  $k_1$ ,  $k_2$  and  $p$  being fixed,  $P + P'$  determines  $Y$  and  $Y$  determines  $P + P'$ .)

Table XVIII.

*Calculated values of  $P + P'$ .*

Superphosphate ( $P$ )	Units of silicate				
	1	2	4	8	16
gm.					
0.0	-0.080	0.161	0.222	0.551	1.003
0.024	-0.068	0.242	0.501	0.765	0.493
0.20	+0.084	0.331	0.435	0.717	0.475
1.00	+0.617	1.188	1.615	3.220	2.158

Subtraction of the amount of superphosphate supplied ( $P$ ) expresses the silicate effect as an "equivalent increment of superphosphate." Division of the calculated value  $p + P + P'$  by the value of  $p + P$  for no silicate expresses the silicate effect in terms of the "relative efficiency of the superphosphate."

Table XIX.

*A. Silicate effect as an equivalent increment in superphosphate.*

Superphosphate ( $P$ )	Values of $P'$ Units of silicate					Mean
	1	2	4	8	16	
gm.						
0.0	-0.080	0.161	0.222	0.551	1.003	0.3714
0.024	-0.092	0.218	0.477	0.741	0.469	0.3626
0.20	-0.116	0.131	0.235	0.517	0.275	0.2084
1.00	-0.383	0.188	0.615	2.220	1.158	0.7596
Mean	-0.168	0.1745	0.387	1.007	0.726	

B. *Effect of silicate on relative efficiency of superphosphate.*Values of  $E$  in terms of unity for  $\text{Si} \equiv 0$ .

Superphosphate gm.	Units of silicate					Mean
	1	2	4	8	16	
0.0	0.803	1.394	1.545	2.355	3.470	1.9134
0.024	0.785	1.508	2.110	2.730	2.090	1.8446
0.200	0.808	1.215	1.387	1.855	1.455	1.3440
1.00	0.726	1.133	1.440	2.578	1.825	1.5404
Mean	0.7805	1.3125	1.6205	2.3795	2.2100	

As would be expected from the random variance of the yields from which these tables are calculated, in both cases the values for any one silicate dressing vary rather widely from one another. The total variation shown by each set of 20 values may be analysed as follows:

Table XX.

*Analysis of variance.*A. *Table of "equivalent increments."*

	Sum of squares	Degrees of freedom	Variance
Total	6.05174	19	
Silicate dressing	3.35156	4	0.83789
Superphosphate	0.82830	3	0.27610
Differential	1.87188	12	0.15599
Superphosphate + differential	2.70018	15	0.18001

$$z' = \frac{1}{2} \log_e \frac{0.83789}{0.18001} = 0.76908$$

B. *Table of "relative efficiencies."*

	Sum of squares	Degrees of freedom	Variance
Total	10.04750	19	
Silicate dressing	6.86404	4	1.71601
Superphosphate	1.06225	3	0.35075
Differential	2.12121	12	0.17677
Superphosphate + differential	3.18346	15	0.21223

$$z' = \frac{1}{2} \log_e \frac{1.71601}{0.21223} = 1.0447$$

In both cases the greater part of the total variation is due to variation in silicate dressing while variation in superphosphate has a much smaller effect. The residue after accounting for the variation due to silicate is however relatively very much smaller for the second table as may be seen by comparing the values of  $z^1$  for the two tables. This table, therefore, which represents the effect of silicate as equivalent to a definite change in the efficiency of the superphosphate present, gives the more adequate account of the data.

A further test of the two alternatives lies in the comparison of the observed yields with those calculated on the assumption that the effect

<sup>1</sup>  $z$  is the natural logarithm of the ratio between the two standard deviations and is used in estimating the significance of any difference between them (v. R. A. Fisher, *loc. cit.* p. 192.)

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of silicate can be represented by the mean figures for "equivalent increment" or for "relative efficiency" given at the foot of Tables XIX, A and B: the two equations are

$$(1) \text{ Yield } (Y) = \frac{1}{\cdot 02922 + \frac{\cdot 0503}{\cdot 406 + p + P'}}$$

where for Si = 0, 1, 2, 4, 8, 16 units,  $P'$  takes values

0, - .168, .1745, .387, 1.007, .726.

$$(2) \text{ Yield } (Y) = \frac{1}{\cdot 02922 + \frac{\cdot 0503}{(\cdot 406 + P) \times E}}$$

where for Si = 0, 1, 2, 4, 8, 16 units,  $E$  takes values

1, .7805, 1.3125, 1.6205, 2.3795, 2.2100.

The calculated yields are given in the following table.

Table XXI.

*Calculated yields: (a) equation (1), (b) equation (2).*

Super-phosphate gm.	Silicate					
	0		1		2	
	(a)	(b)	(a)	(b)	(a)	(b)
0.0	6.52		4.15	5.31	8.60	8.08
0.024	6.84		4.52	5.57	8.89	8.44
0.200	8.90		6.92	7.38	10.68	10.81
1.000	15.38		14.31	13.30	16.40	17.69
5.000	25.99		25.72	24.25	26.18	27.55
	4		8		16	
	(a)	(b)	(a)	(b)	(a)	(b)
	(a)	(b)	(a)	(b)	(a)	(b)
0.0	10.80	9.46	15.42	12.30	13.60	11.72
0.024	11.02	9.84	15.60	12.72	13.77	12.16
0.200	12.52	12.41	16.58	15.60	14.96	14.96
1.000	17.32	19.50	19.98	22.58	18.94	22.00
5.000	26.40	28.60	27.00	30.18	26.70	29.88

For each equation 5 constants additional to the original 3 have been calculated from the data, so that for the estimation of the variance of deviations from the expected yields we have, for the set of treatments silicate 0-16 units and superphosphate 0-1.0 gm., 24-8 = 16 degrees of freedom. The variance of deviations (sum of squares divided by the number of degrees of freedom) for the first equation is 3.8754, for the

second it is 2.7533. The random variance of parallels for this group of pots is 2.0607 (72 degrees of freedom) so that for the first case

$$z = \frac{1}{2} \log_e 3.8754/2.0607 = .3156,$$

for the second  $z = \frac{1}{2} \log_e 2.7533/2.0607 = .1448$ .

The value of  $z$  required for a probability of .05 is about .289<sup>1</sup>, so that the deviation between observed and calculated yields in the first case is greater than would be expected by chance once in twenty trials and must be considered significant. The deviations from the second equation are greater than the random deviations, but not significantly so, and this equation may be accepted as an approximate representation of the observed facts.

So far we have dealt only with the yields which have been used in calculating the constants of the equation. We may calculate also yield values for complete superphosphate and silicate. (These are given in the lowest row of Table XXI.) The variance of deviations (for the second equation) now becomes 3.6805 (22 degrees of freedom) while that of parallels is 2.4228 (90 degrees of freedom). For these variances  $z = 0.209$  while for a probability of .05 the value of  $z$  required is 0.2537 so that the deviations are still not significant.

Of the whole experiment we are now left with the no-potash and the no-manure series. If these show the same silicate-phosphate interaction as the other series the same equation should serve with an alteration only in the constant  $k_1$  which is not associated with the silicate-superphosphate function.

For the no-potash set calculation gives  $k_1 = .031404$  instead of .02922 and for the calculated yields we have (for Si = 0, 1, 2, 4, 8, 16), 24.043, 22.661, 25.400, 25.912, 27.205, 27.068. The variance of deviations of this series 2.7182 (with 5 degrees of freedom) is less than the variance of parallels which is 3.0173. For the whole experiment, excluding the no-manure series, we have the variance of deviations 3.5023 and the variance of parallels 2.5219 (108 degrees of freedom). For these variances  $z = .1647$ , whereas for a probability of .05  $z$  requires to be = .2314. Thus the slight observed effect of silicate upon yield in the absence of potash, can be represented as the effect of the silicate upon the efficiency of the superphosphate supplied and no sodium-potassium interaction is suggested.

The value of  $k_1$  for the no-manure series is calculated as .03837 and the calculated yields (for Si = 0, 1, 2, 4, 8, 16) using this constant are

<sup>1</sup> Using the approximation formula given by Fisher, *loc. cit.* p. 199.



6.159, 5.066, 7.526, 8.705, 11.041, 10.585. The greater part of the variance in the no-manure series is thus accounted for, but the variance of deviations remains high and in comparison with the very low value of the variance of parallels is fully significant. The variance of deviations (5 degrees of freedom) is 2.2628; the variance of parallels (18 degrees of freedom) is 0.56305. Thus  $z = .69564$  while for a probability of .05,  $z$  is about 0.51. The chief divergence between the expected and the observed values lies in the absence from this series of the depression in yield with one unit of silicate shown by the 5 manures containing both nitrogen and potash. For the purpose of fitting the general equation for the silicate-phosphate interaction this depression was accepted as part of the data from which constants for the effect of the different doses of silicate were to be derived. Tested statistically the mean depression in yield for unit dose of silicate is just on the verge of significance. For  $Si = 0$  the mean yield for pots with N and K and varying P is 12.9558; for the same manures with  $Si = 1$  unit the mean yield is 11.1252. The mean difference is 1.8306 while the level of significance ( $\sigma m_1 - m_2 \times t$  for a probability of .05) for this comparison is very little greater—1.99.

It is just possible therefore that the apparent depression in yield with unit dose of silicate may not be real. In fact the error in representing the mean value of  $E$  (Table of Relative Efficiencies, p. 77) as a linear function of amount of silicate is, up to  $Si = 8$ , less than the error due to differential response. Beyond  $Si = 8$ , there may be a depression or not: the data again are inadequate for an exact decision.

Up to  $Si = 8$  units however a reasonable account of the results in terms of concentration of superphosphate and concentration of silicate may be obtained from an equation of the type we have been discussing:

$$Y \text{ (Yield)} = \frac{1}{k_1 + \frac{k_2}{(p + \bar{P})} E},$$

where  $k_1 = .02922$  for manuring with N and K,  
            $= .031404$  for manuring without K,  
            $= .03837$  for manuring without N or K,

$k_2 = .0503$ ,  $p = .406$ ,

$P =$  gm. of superphosphate supplied as manure and  $E$  (representing the efficiency of the superphosphate)  $= .8403 + .192 \times Si$ , where  $Si =$  no. of units of silicate supplied.

This involves six constants derived from the yield data. For the variance

of deviations of the 35 yields up to  $Si = 8$  we have therefore 29 degrees of freedom and the variance is  $82.7097/29 = 2.85206$ . The variance of parallels (105 degrees of freedom) is 2.10324. Hence  $z = 0.15228$ , while the value of  $z$  required for a probability of .05 is about .227.

Thus within the limits set by the variation of parallel pots it seems possible to formulate the effect of added silicate in terms of an increase in the efficiency of the superphosphate present, this increase in efficiency approximating to a linear function of amount of silicate up to 8 units of silicate.

#### D. SUMMARY.

(1) Under controlled conditions in water cultures soluble silicate was found to have little effect upon the growth of barley if phosphorus were also present, but if the latter were absent a significant increase in dry weight was induced by the silicate.

(2) The addition of silicate caused an appreciable increase in the height of the main shoot, which was most marked in phosphate-free solutions, becoming less evident as the quantity of phosphate present was increased.

(3) Leaf development was retarded by phosphate deficiency and hastened by the addition of silicate.

(4) A close association exists between the amount of phosphate present, and the effect of silicate upon the rate of tillering and the number of tillers developed.

(5) Soil cultures with barley and mustard in pots with various forms of silicate showed that soluble silicates are more active than glass silicates, tending to cause increase in dry weight with deficient mineral manuring, and in some cases also with complete manuring.

(6) Further soil experiments revealed variations in the response of barley and mustard to silicate on different types of soil. A general improvement occurred with increasing doses of silicate together with various combinations of manures, notably when phosphorus or potash was deficient.

(7) The significance of the results obtained has been examined statistically, and an attempt made to formulate the effect of added silicate in terms of an increase in the efficiency of the superphosphate present.

## E. BIBLIOGRAPHY.

- (1) CRÜGER, H. (1857). Westindische Fragmente. Neuntes Fragment. El Cauto. *Bot. Zeit.* xv, 281-92, 297-309.
- (2) DENSON (1924). Versuche über den Einfluss von leicht löslicher Keiselsäure auf den Ertrag und die Ausnutzung der Phosphorsäure. *Landw. Jahrb.* LX, 142-5.
- (3) GLE, P. L. and SMITH, J. G. (1925). Colloidal silica and the efficiency of phosphate. *Journ. Agric. Research*, xxxi, 247-60.
- (4) FISHER, R. A. (1925). *Statistical Methods for Research Workers*. London. Table IV.
- (5) FLICHE, P. (1889). Recherches chimiques et physiologiques sur la famille des Ericacées. *Revue des eaux et forêts. Engl. Journ.* xiii, 12.
- (6) HALL, A. D. and MORISON, G. C. T. (1906). On the function of silica in the nutrition of cereals. Pt. 1. *Proc. Roy. Soc. B*, LXXVII, 455-77.
- (7) HATTENSAUR, G. (1890). Zur chemischen Zusammensetzung von *Molinia caerulea* (Mönch) von Königsberg bei Raibl. *Ber. der Kais. Akad. der Wissenschaft, Wien*, xcix, II b, 29-31.
- (8) JENNINGS, D. S. (1919). The effect of certain colloidal substances on the growth of wheat seedlings. *Soil Science*, vii, 201-15.
- (9) LEMMERMAN, O. and WIESSMANN, H. (1922). Die ertragssteigernde Wirkung der Kieselsäure bei unzureichender Phosphorsäureernährung der Pflanzen. *Ztschr. Pflanz. Düng.* A 1, 185-255.
- (10) LEMMERMAN, O. WIESSMANN, H. and SAMMETT, K. (1925). Untersuchungen über die Ursache der ertragssteigernden Wirkung der Kieselsäure. *Ztschr. Pflanz. Düng.* A 4, 265-315.
- (11) MASKELL, E. J. (1925). A note on the Yield Factor Relation; Ph.D. Thesis. Cambridge University Library.
- (12) NANJ, D. R. and SHAW, W. S. (1925). The rôle of silica in plant growth; its assimilation and physiological relation to phosphoric acid. *Journ. Soc. chem. Ind.* XLIV, 1-6 T.
- (13) OTT, E. (1900). Beiträge zur Kenntnis der Harte vegetabilischer Zellmembran. *Oesterreich. bot. Zeitsch.* Jahrg. L, No. 7, 237-241.
- (14) PFEFFER, W. (1900). *Physiology of Plants* (English trans. by A. J. Ewart), I, 416, 435; II, 33, 55.
- (15) PIERRE, I. (1866). La silica et la verse des blés. *Compt. Rend.* 374-7.
- (16) RICHARDSON, W. D. (1920). The ash of dune plants. *Science*, N.S. LI, No. 1326, 546-51.
- (17) SCHOLLENBERGER, C. J. (1922). Silica and silicates in relation to plant growth and composition. *Soil Science*, xiv, 347-61.
- (18) WICKE, W. (1862). Ueber das Vorkommen und die physiologische Verwendung der Kieselerde im Pflanzenreiche. *Bot. Zeit.* xx, 76-9.
- (19) WOLFF (1871, 1880). Aschenanalysen.

## EXPLANATION OF PLATE V

- Fig. 1. Barley grown in nutrient solution containing no phosphate, with sodium silicate ranging from 0-16 units (1st harvest).
- Fig. 2. Barley grown in complete nutrient solution, with sodium silicate ranging from 0-4 units (1st harvest).
- Fig. 3. Barley grown on light soil with sulphate of ammonia and superphosphate, but no potash, with sodium silicate ranging from 0-16 units, left to right.
- Fig. 4. Mustard grown on light soil with sulphate of ammonia and potash but no phosphate, with sodium silicate ranging from 0-16 units, left to right.

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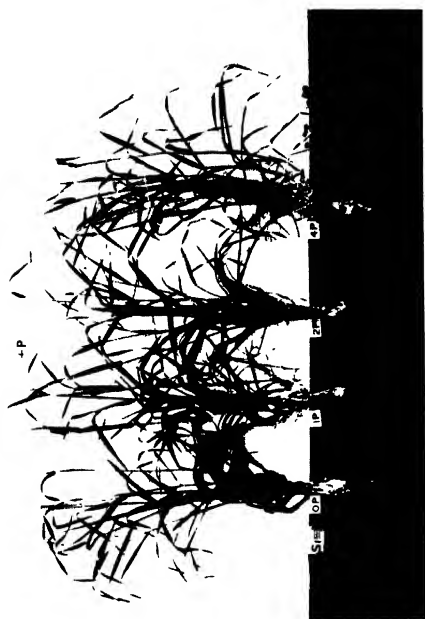


FIG. 2.

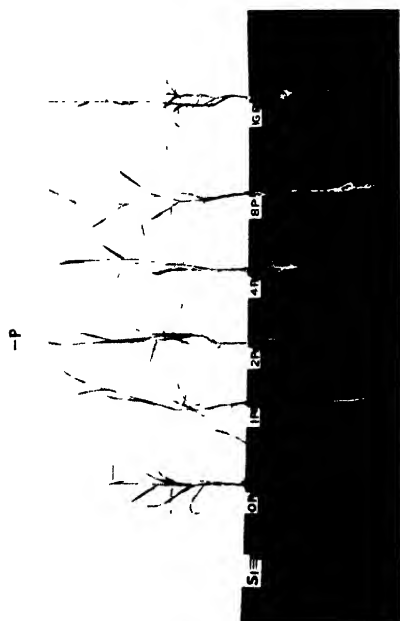


FIG. 1.

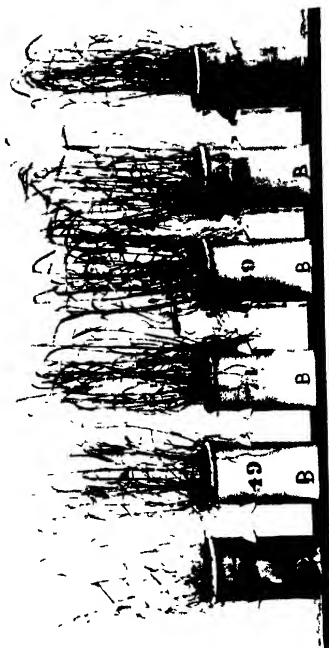


FIG. 3.



FIG. 4.

discriminate between the effect of eliminating bunt and other influences which the chemical as such might possibly have on the growth of the plant.

In addition to the superior early growth of untreated rows, it appeared on a superficial examination that healthy plants were longer in the straw than those carrying bunted heads, and since little attention had previously been directed to the influence of the fungus on the growth of the plant, the authors decided to make a critical comparison of the establishment, tillering, growth in height and final yield of healthy and bunted plants of the same variety of wheat, reserving as a distinct and separate problem the influence of treatment with chemicals.

The problem was studied during the years 1924-5 by three methods:

(1) Establishment and yield data were obtained from rows of bunt-free and bunt-contaminated grain, sown under field conditions in the cage at the Plant Breeding Station Farm. (Experiment I.)

(2) Germination and dry-weight data were collected from seedling plants grown in boxes under varied conditions of soil and temperature. (Experiment II.)

(3) Measurements were made throughout the entire growth period on single plants, bunted and healthy. The plants in this case were grown in pots in the cage at the Plant Breeding Station Gardens. (Experiments III and IV.)

The results prove that bunt has a retarding influence on growth in height, which under certain conditions manifests itself at an early stage in the development of the plant. Tillering, on the other hand, appears to be stimulated by the parasite, bunted plants showing on the average a higher number of tillers per plant than those which are healthy. This result is of particular interest in view of the data from field trials previously referred to, in which treated rows carried a far higher number of heads at maturity than those not treated, but the apparent discrepancy in results may be explained by the failure, under certain conditions, of bunt-contaminated seed to establish a good stand in the field, a result obtained with three varieties in 1925. (Experiment III.)

It is evident that bunt has a more retarding effect on growth and yield than has been generally realised, and the remarkably high increase in grain yield following its control in field experiments can now be explained without resort to the hypothesis of a stimulating effect on the part of chemicals applied to the grain. There is, however, still the possibility that treatment does also influence growth in this way, or in preventing the attacks of soil organisms during the germination period,

and the question of the effect of various chemicals on the establishment and growth of bunt-free wheat samples is the subject of a separate investigation. Data on the growth of grain treated with copper carbonate are, however, given in one experiment.

## II. HISTORICAL.

The early investigations on bunt of wheat, prior to the year 1921, have been summarised by Woolman and Humphrey (16), and it is noteworthy that little attention has been given to the effect of the fungus on the host plant apart from the final production of spores in place of healthy grain.

That bunt has a retarding influence on the development of the wheat plant was noted by Lang (8) in 1917. A dwarfing effect due to *T. tritici* was described by Harwood (4) and other workers (11) and it was suggested that this character might be made use of for distinguishing the two species *T. tritici* and *T. laevis* in the field since the latter fungus appeared to influence the growth of the plant only to a very slight extent.

Various references have been made to morphological differences between the ears of healthy and bunted plants. A lengthening was recorded for ears of the squarehead (2) and *compactum* types (6, 9, 15) but in a spring bearded wheat no difference in length was apparent between healthy and bunted heads (13). Some measurements on ears and kernels, healthy and bunted, were given by Barrus (1), but the first worker to record data estimating the influence of bunt on growth in height was Mourashkinski in 1925 (10). Both species of *Tilletia* were included in his experiments, which consisted of field plots of bunt-free and bunt-contaminated samples of the same variety sown at spaced intervals and left until maturity when they were taken up for measurement. The data show conclusively that the infected plants were shorter in each internode than plants raised from healthy seed, while plants with healthy ears raised from contaminated seed occupied an intermediate position.

In 1924, using *Triticum vulgare* var. *milurum*, the depressing effect of the two species of *Tilletia* was equal, but in 1925 it was slightly more marked in the case of *T. tritici*, although the same pure line of wheat was used as in the previous year. It is suggested that the difference between the two sets of data may possibly be attributed to the fact that growth conditions were not so favourable for wheat in 1924, or perhaps to a difference in the spore collections of *T. laevis* used in the two seasons.

In several experiments counts were made in the field of seedlings established in plots sown respectively with healthy and bunt-contaminated grain. Under some conditions it was found that the mortality was distinctly higher in the case of bunted grain, but the degree of mortality varied with the variety and with the time and conditions of sowing the grain. A higher percentage of mortality was actually found in the case of certain varieties recorded on the basis of plants with bunted heads as less susceptible.

Discussing the actual cause of mortality among bunted plants, the author refers to the fact that such plants are more than ordinarily susceptible to attacks of *Fusarium* sp.—a fungus causing considerable damage to wheat in the east of Russia.

In addition to a shortening of the stem internodes, bunted plants showed in the majority of varieties a reduction in the length of ear and in the number of spikelets produced, but three varieties gave contrary results. Where such reduction did occur it was manifest also in healthy ears raised from contaminated seed.

Finally, data are given on the size of bunted grain, and a certain correlation is found to exist between this character and the susceptibility of a variety to bunt, the bunted grains being longer than the normal in the more susceptible varieties and shorter than the normal in those that are more resistant.

The experiments described below carried out on somewhat different lines and with different wheat varieties confirm in all essential points the results obtained in Russia. In addition, data are given on the progressive tillering of bunted and healthy plants, and on the influence of *Tilletia tritici* on the height and the dry weight of bunted and healthy plants in the seedling stage.

### III. EXPERIMENTAL DATA.

(1) Establishment and yield data from rows of bunt-free and bunt-contaminated grain sown under field conditions. (Experiment I.)

Varieties: Browick, Marshal Foch, Svalöf Iron. Sown February 18th, 1925.

April Bearded, Red Marvel. Sown April 9th, 1925.

Replications: Eight 5-ft. rows of each treatment for each variety.

The object of this experiment was two-fold: (i) to compare the behaviour of healthy and bunted grain of the same variety grown under exactly similar conditions; (ii) to study the effect of the copper carbonate treatment on grain free from bunt.

Since sufficiently large samples of grain of known origin were not available, it was necessary to obtain new samples from a seed firm. Of the five varieties included in the experiment, four were apparently bunt-free, in that the grain sown without treatment did not produce a single bunted head. The fifth sample, April Bearded, was evidently bunt-contaminated on receipt, the control rows containing at harvest an average of 9.7 per cent. bunted heads.

The five varieties received uniform treatment, except in regard to the dates of sowing, which varied with the winter and spring varieties.

Each bulk sample (1 lb.) was divided into four lots which received the following treatment:

- A. Control—grain as received.
- B. Grain as received, shaken with copper carbonate at the rate of 2 oz. per bushel.
- C. Grain as received, shaken with bunt spores at the rate of 1 gm. spores per 100 gm. grain. The spores were derived from the variety April Bearded in 1924, the bunted grain being kept in a tin out-of-doors from October to February, then crushed and sieved.
- D. Half of the contaminated sample (C) was treated with copper carbonate at the same rate as that used for the healthy grain, sample (B).

The varieties were sown at a uniform rate, 120 grains per 5-ft. row and each treated lot was replicated eight times.

The data collected from the four varieties in which the control rows were entirely bunt-free, are given in Table I, while those obtained from April Bearded are shown separately in Table II.

### *Discussion of Results.*

The winter varieties sown in February were subject to highly unfavourable climatic conditions during the first seven weeks, with the result that germination was extremely slow and uneven<sup>1</sup>. When critically examined in May a marked difference was evident between the rows, the control of each winter variety having made a distinctly better stand than the bunt-contaminated lots. Rows from grain treated with copper carbonate (B) were not quite so good as the control (A), but rather better than those in which the copper dust had been applied to bunt-

<sup>1</sup> The following data were kindly supplied by Mr Martin G. Jones, M.Sc., meteorological observer at the Welsh Plant Breeding Station: For the week ending March 1st, 1925, the total rainfall for the week was 2.14 in., some rain fell each day and the minimum temperature ranged from 32 to 39° F. During the first seven weeks after sowing (February 18th, 1925) twenty-one days showed ground frost and rain fell on twenty-eight days.



contaminated grain (D). The rows were graded on a system of marking in which a maximum of five marks was allocated to a thick uniform row and two days later actual counts were made on each row in the experiment, the two sets of data showing close agreement. (Table I.)

The two spring varieties sown early in April had already germinated and made a uniform stand when the first count was made, no difference appreciable to the eye being evident between the rows. In actual figures the lots treated with copper carbonate showed in both varieties slightly better establishment than the control or the bunt-contaminated rows.

Comparing the healthy control (A) rows with those containing bunted heads in regard to the number of plants established in May, the number found at maturity and the average number of heads per row at harvest, a progressive increase in the influence of bunt is evident, amounting to 16, 19 and 25 per cent. for the three sets of data in the case of lots badly bunted (B); 14, 15 and 17 per cent. in the case of lots where bunt was partially controlled by the copper dust treatment (D). (Table I.)

The retarding influence of bunt on growth in height was especially conspicuous at the "boot stage," just prior to emergence of the spike. Measurements were made in each row at three points, situated approximately in the middle, and at a foot from the two ends. The distance measured in the one case was from ground level to the tip, and in the other, to the ligule of the flag leaf of the tallest tiller at each place of measurement. The results show for each variety an appreciable reduction in the height of bunted rows, although the measurements were not made exclusively on plants showing bunted heads since such plants could not be recognised at that stage of growth.

Considering the grain yield data we find here also a confirmation of the results of previous field experiments. Taking the average of four varieties the percentage loss in grain weight is 64, while the percentage bunted heads is 57. The difference between the two figures is no doubt due to the lower rate of establishment of the bunt-contaminated grain.

It is not the purpose of the present paper to treat fully of the influence of chemicals on the growth of wheat, but reference might be made in this connection to the series (A) and (B), which show in all the data collected remarkably close agreement. From this experiment, therefore, it cannot be said that the copper dust has definitely affected either beneficially or adversely the healthy grain, whereas the retarding influence of bunt is seen at all stages in the growth period.

A further point of interest in the experiment is the relatively high percentages of bunt obtained in the lots treated with copper carbonate,

Table I.

*Showing establishment, height and yield of four wheat varieties, bunted and non-bunted, treated with copper carbonate. Winter varieties sown February 18th, 1925. Spring varieties sown April 9th, 1925. Knoll cage.*

Treatment and variety	Bunted plants %	Bunted heads %	Marks for braiding. Max. 5 20. v. 25	No. of plants established 22. v. 25	No. of plants at maturity average per row	Height to tip of flag leaf 16. vi. 25 cm.	Height to ligule 26. vi. 25 cm.	No. of heads per row at maturity	Total weight straw + grain gm.	Weight of healthy grain gm.	Yield of healthy grain: control at 100
A. Control: grain as received.											
Browick	0.0	0.0	2.0	19.2	25.1	59.3	54.8	58.9	310.8	92.8	100
Marshal Foch	0.0	0.0	2.8	21.4	27.4	59.9	57.0	62.9	325.8	117.3	100
Svalof Iron	0.0	0.0	3.8	39.5	54.8	57.6	51.3	80.1	358.5	129.6	100
Red Marvel	0.0	0.0	5.0	57.4	58.2	57.8	56.6	81.8	269.8	81.3	100
Average	0.0	0.0	3.4	34.4	41.4	58.7	54.0	70.9	316.2	105.2	100
B. Copper carbonate on grain as received:											
Browick	0.4	0.2	1.8	18.4	23.5	57.1	52.7	57.3	294.9	85.2	92
Marshal Foch	0.0	0.0	2.1	19.9	26.0	61.5	56.4	65.8	329.6	122.4	104
Svalof Iron	0.0	0.0	3.4	36.1	44.1	57.1	52.5	74.4	325.6	113.6	88
Red Marvel	0.8	1.0	5.0	62.5	67.0	58.4	56.4	98.8	306.9	94.2	116
Average	0.3	0.4	3.1	34.2	40.2	58.5	54.5	74.1	314.2	103.9	100
C. Contaminated with bunt spores:											
Browick	53.1	12.6	1.1	12.1	15.1	53.2	46.8	30.6	169.6	42.1	45
Marshal Foch	43.9	43.1	1.6	15.2	22.4	53.0	48.2	42.4	177.2	54.8	47
Svalof Iron	54.9	51.3	2.5	29.5	36.6	52.4	46.0	51.8	185.2	53.9	42
Red Marvel	92.7	91.4	5.0	58.6	58.4	52.3	49.3	80.8	162.2	7.8	10
Average	61.2	57.1	2.6	28.9	33.1	52.7	47.5	52.9	173.6	39.7	36
D. Contaminated with bunt spores and treated with copper carbonate:											
Browick	8.0	4.2	1.5	13.9	17.8	57.3	51.6	45.0	261.6	79.7	86
Marshal Foch	10.1	6.1	2.1	15.4	22.3	62.3	54.4	52.4	254.4	88.0	75
Svalof Iron	13.8	12.8	2.1	25.6	32.4	53.2	54.4	54.8	241.6	85.3	66
Red Marvel	41.6	38.4	5.0	63.4	68.2	57.8	56.8	84.5	237.4	62.5	76
Average	18.4	15.4	2.9	29.6	35.2	57.7	54.3	59.2	198.8	78.9	76

Table II.

*Showing data on establishment, growth and yield of April Bearded wheat with varying amounts of bunt. Sown April 9th, 1925. Knoll cage.*

Treatment	Bunted heads at harvest %	Av. number of plants per row 22. v. 25	Av. height to tip of flag leaf 16. vi. 25 cm.	Av. height to highest ligule 26. vi. 25 cm.	Av. number heads per row at harvest	Total weight straw + grain gm.	Weight of healthy grain gm.
Grain as received	9.7	66.1	83.1	65.6	112.4	323.6	95.8
Copper carbonate on grain as received	0.9	71.5	75.5	67.3	127.9	371.9	114.3
Contaminated with bunt spores	93.1	66.4	64.2	57.3	112.5	193.0	7.0
Contaminated and treated with copper carbonate	52.7	72.0	72.5	63.0	131.9	313.2	57.6

but it should be understood that the rate of spore contamination, 1 per cent., was excessively heavy and one hardly like to occur under practical conditions<sup>1</sup>. This may also account in part for the unusually high percentage of bunt obtained with spring-sown wheat which is commonly held to be less heavily attacked than wheat sown in the autumn.

(2) Germination and dry weight data obtained from seedling plants under varied conditions of soil and temperature.

Variety: Marshal Foch. Sown November 17th, 1925. (Experiment II.)

For this experiment a clean bulk sample was divided into two portions, one of which was kept as the control, while the other was shaken with spores of *T. tritici*, known to be viable. The grain was sown at spaced intervals in boxes measuring  $21 \times 12 \times 7$  in. and covered to a uniform depth. Each box carried 104 grains. In half of the total number of boxes (36) partially sterilised soil was used, the remainder were filled with soil which had not been sterilised<sup>2</sup>. The boxes were divided between three places of experiment, a heated glass-house, a cold glass-house and an open cage, in order that the plants might be subject to different conditions of temperature. The experiment thus included twelve series, each consisting of three replications. All the boxes were sown and placed in position on the same day. Notes and counts on the rate of germination were made at intervals, and in the cold house, where a decided difference in height was apparent between the "healthy" and "bunted" series, the individual plants in each box were measured from soil level to the apex of the longest leaf. Dry weight data were finally obtained by carefully removing the plants from each box and separating shoots from roots by cutting the hypocotyl at the point where the grain was still attached. Except in the case of those situated in the heated house, which were the first to be taken up, the shoots were dried and weighed in lots of twenty plants, and the weight per 100 was calculated on this basis. The roots were also washed, dried and weighed, but the data have been omitted from Table III as the experimental error was relatively high and no definite conclusion could be drawn from the results.

<sup>1</sup> In experiments conducted by Heald (5) a definite relation was found to exist between the spore load carried by the grain and the percentage disease appearing in the crop. Maximum smutting was produced when 0.5 gm. of spores were applied per 100 gm. grain.

<sup>2</sup> Partial sterilisation was achieved by heating the soil in a brick oven. The soil was first saturated with water and heated to 85–90° C., this temperature being maintained for approximately 12 hours.

Table III.

*Showing a comparison between the early growth of plants raised from bunt-free (control) and bunt-contaminated grain and sown under varied conditions of soil and temperature. Variety: Marshal Foch. Sown November 17th, 1925.*

Place of experiment	Age of seedlings when taken up weeks	Germination in soil, %		Av. dry weight of shoots per box in gm.		Av. dry weight of shoots per 100 plants in gm.		Av. height per plant in cm.	
		Control	Bunt-contaminated	Control	Bunt-contaminated	Control	Bunt-contaminated	Control	Bunt-contaminated
Partially sterilised soil:									
Heated glass-house	8	92	90	5.853	4.900	6.096 (100)	5.276 (87)	—	—
Cold glass-house	17	98	98	7.305	6.049	7.141±.076 (100)	6.002±.021 (84)	22.2±.110 (100)	18.5±.137 (83)
Open cage	21	97	95	17.154	17.173	16.940±.243 (100)	17.149±.213 (101)	—	—
Non-sterilised soil:									
Heated glass-house	8	88	92	4.230	4.380	4.640 (100)	4.560 (98)	—	—
Cold glass-house	17	78	73	4.632	3.465	5.732±.108 (100)	4.558±.097 (80)	18.8±.176 (100)	16.2±.221 (86)
Open cage	21	80	80	4.866	5.816	5.912±.249 (100)	6.920±.271 (117)	—	—

### *Discussion of Results.*

The different temperature conditions to which the plants were subject are indicated by the rate of germination in the three places of experiment. In the heated glass-house an average of 22 plants per box were above soil level on November 26th, only nine days after sowing, and the maximum establishment figure was obtained on December 11th. On this date germination in the cold glass-house was only just apparent, while in the open cage not a single seedling was in view. In this situation no plants appeared above soil for five weeks from the date of sowing. During this period the temperature fell below freezing point on nineteen days and the actual range for the same period was maximum 36–45° F., minimum 18–40° F.

In regard to the percentage germination no constant difference was apparent between the bunt-contaminated grain and the bunt-free control. The greatest difference was a 5 per cent. decrease in the cold house (non-sterilised soil), but in the same soil, in the heated house, a 4 per cent. increase was recorded for the same grain. Taking the average of all boxes in the three places of experiment the results are 94 and 96 per cent. for bunt-contaminated and bunt-free grain respectively in the partially

sterilised soil and in the non-sterilised soil 82 per cent. germination was recorded for both lots of grain.

In later growth, however, there was a distinct and visible difference between the control and the bunt-contaminated grain in both soils in the cold house as is shown by Plate VI (2) and (3).

By measurement the difference in height in favour of the control plants was found to amount to 14 and 17 per cent. for the non-sterilised and partially sterilised soils respectively. A distinct and significant difference in the same direction was obtained in the dry weight of shoots per 100 plants. In non-sterilised soil the control shoots were 20 per cent., in partially sterilised soil 16 per cent. heavier than those derived from bunt-contaminated grain.

In the heated house, and in the open cage, no visible difference could be detected between the two lots of seedlings in either type of soil. Plate VI (1) and (4) shows the similarity of the two series in height and general growth, and it was not thought useful to make actual measurements. In regard to the dry weight of shoots per 100 plants a 13 per cent. decrease was shown by the plants raised from bunt-contaminated grain in partially sterilised soil, but from the range of figures it is doubtful if the difference is significant, and it was not confirmed in the other type of soil.

In the open cage again no reliable difference was evident. Apart from the low temperature the plants here were subject to extremely severe conditions of wind and rain, and this increased the accidental differences between boxes in the same series. The apparently large difference (17 per cent.) in favour of the bunted lots in the dry weight of shoots per 100 plants is without mathematical significance.

In this experiment, therefore, a retarding effect due to bunt was distinctly shown at an early growth stage under one set of conditions, but was absent when the same seed was grown under two different sets of conditions.

A somewhat similar result was obtained in another experiment. It was noticed in February in a field trial that rows of seedlings raised from bunt-contaminated grain treated with copper carbonate were far superior in appearance to others of the same seed not treated. Since samples of both lots of seed were still available, 416 grains from each sample were sown in boxes which were placed in the heated glass-house. Dry weight data were obtained after eight weeks as in the above experiment, but no appreciable difference in shoot development existed between the two lots, thus giving a result contrary to that obtained under field conditions.

A slight difference between the two lots was, however, shown in the root data:

	Dry weight shoots only		Dry weight roots only	
	Average	Range	Average	Range
Bunt-contaminated grain	2.665	(2.09-3.87)	0.333	(0.29-0.39)
Grain treated with copper carbonate	2.655	(2.31-2.96)	0.392	(0.33-0.49)

It is possible that the similar behaviour of the two samples in the heated house was due to the fact that the fungus failed to infect the plants under the temperature conditions there prevailing, since it is well known that a low temperature favours infection of wheat by this fungus (3). Unfortunately this point could not be tested by growing on the plants to maturity. On the other hand, in experiments described below the retarding influence of bunt on growth was evident at later growth stages, but inappreciable in the young plant, and its absence at that stage does not of necessity mean that plants are free from infection.

It has not been possible to investigate more fully this apparent correlation between temperature conditions and the influence of bunt on growth. Reference might be made to the previous section of this paper in which it was shown that the establishment of bunt-contaminated grain was lowered in the case of three varieties sown in February while the effect was absent in the case of two other varieties sown in April. Mourashkinski recorded the fact that bunt-infected plants may die before maturity and discussing the problem states that the percentage of death varied with several factors, including the time of sowing.

(3) Measurements made throughout the entire growth period on single plants, raised from bunt-contaminated and bunt-free grain.

Varieties: Hen Gymro, pure line selection. Sown March 14th, 1924.  
(Experiment III.)

April Bearded. Sown March 4th, 1925. (Experiment IV.)

The method adopted with the variety Hen Gymro in 1924 was briefly as follows. The bulk sample of grain was first sterilised by soaking it for ten minutes in formalin solution (1 part in 320 parts of water) and covering it for four hours with a cloth soaked in the same solution. The grain was then thoroughly washed with tap water and finally spread out to dry. Before sowing, the sample was separated into grain of two sizes, and the average weight of each lot was determined. For the experiment each grain sown was weighed separately and only those were included which showed a difference of less than 12 per cent. from

the average weight of the grain in the particular series. Each lot of selected grain was further subdivided, one half being reserved for the control, and the other half contaminated by shaking with spores of *Tilletia tritici*. The experiment thus consisted of four series:

(1) Small grain bunt-free (control)	...	...	8 pots
(2) „ „ bunt-contaminated	...	...	17 „
(3) Large „ bunt-free (control)	...	...	8 „
(4) „ „ bunt-contaminated	...	...	17 „

Each series was sown on March 14th, 1924, four grains being allowed for one pot (tomato size) with the intention of allowing three to reach maturity. In a few instances it was necessary to make up the number in a particular pot by transplanting seedlings from one pot to another in the same series. This was carefully done at a very early stage of growth without any apparent check on the growth of the plant.

Measurements on height were made at weekly intervals during the growing season and at the same time counts were made on the tillers produced by each plant.

At maturity each plant was taken up, wrapped round with paper and classified, after examination in the laboratory, as healthy, semi-bunted or completely bunted, according to the condition of the grain. Only those plants were placed in the last group when the heads failed to show a single healthy grain. Data were also obtained for each plant on the number of heads, the weight of straw and the height of each tiller

Table IV.

*Showing the relative tillering and height of single plants of wheat raised from bunt-free and bunt-contaminated grain. Variety: Pure line selection Hen Gymro. Sown March 14th, 1924.*

Variety	No. of plants averaged	No. of heads at maturity per single plant	Height to base of ear cm.	Height to apex of ear cm.	Length of ear (by difference) cm.	Weight of straw per plant gm.
Small grain. Contaminated ( <i>T. tritici</i> ):						
Not-bunted	10	7.8	103.9	115.2	11.3	17.2
Semi-bunted	21	7.0	89.7	100.2	10.5	12.3
Completely-bunted	12	8.9	80.2	89.8	9.6	11.9
Control	23	7.7	98.8	109.4	10.6	16.9
Large grain. Contaminated ( <i>T. tritici</i> ):						
Not-bunted	8	6.9	102.3	114.6	12.3	15.4
Semi-bunted	28	8.7	91.5	103.1	11.6	18.0
Completely-bunted	11	8.7	95.6	94.8	9.2	14.9
Control	23	7.5	102.5	115.3	13.2	16.7

measured from the level of the root system to the base and the apex of the ear. The results are given in Table IV.

The experiment was repeated in the following year, using April Bearded as the variety of wheat, and including the species *Tilletia laevis* in addition to *T. tritici*<sup>1</sup>. The method of carrying out the experiment was essentially the same as in the previous season with one or two slight modifications. The sample was graded as before, but only grain of one "size" was used, namely that which fell between .025–.035 gm. in weight. Heated soil was used as a few plants had been lost in 1924 by wireworm attack, and six grains were sown per pot to guard against the necessity of transplanting. These were finally thinned to four as it was found that such a number could reach maturity without becoming pot-bound. The experiment thus consisted of the following series:

- |   |          |
|---|----------|
| (1) Bunt-free seed (control) ... ..                           | 10 pots. |
| (2) Seed contaminated with spores of <i>T. tritici</i> ... .. | 20 "     |
| (3) " " " <i>T. laevis</i> ... ..                             | 10 "     |

As in the previous experiment, data on tillering and growth in height were collected during the growing season and at maturity the plants were carefully lifted and tied in paper. In addition to measurements on length of straw, number and weight of heads and grain, dry weight data were obtained on the roots, which were carefully freed from soil and then washed and dried. The root system was severed by cutting across the base of the plant at a point level with the highest root on the stem. The results are given in Table V.

### *Discussion of Results.*

In the 1924 experiment, using Hen Gymro wheat, the "small" grain gave 77 per cent., the "large" grain 83 per cent. of bunted plants. In regard to height of straw and length of ear the average figures for plants raised from the large-sized grain were slightly higher than those obtained by sowing the smaller seed, but as far as the influence of bunt on growth is concerned the two series gave closely parallel results. Thus it is evident (Table IV) that in both series bunted plants when compared with the control showed a decided reduction both in height (16–19 per cent.) and in the length of the ear (9.4–30.3 per cent.). The reduction was, however, less in plants not completely bunted.

In 1925 a similar reduction (amounting to 13 per cent.) in the length of straw was obtained with *T. tritici*, on April Bearded wheat, but no appreciable difference was apparent in the length of ear, the average

<sup>1</sup> Spore collection of *T. laevis* was derived from the variety Poole. It was placed at the authors' disposal by the kindness of Dr G. H. Pethybridge.



for bunted plants being very slightly higher than that for the control. The diseased plants in this experiment showed in almost every case a certain number of healthy grain, and therefore no classification could be made into groups of semi-bunted and completely-bunted individuals.

Table V.

*The relative growth and yield of single plants of wheat raised from bunt-free and bunt-contaminated grain. Variety: April Bearded. Sown March 4th, 1925.*

Treatment	No. of plants averaged	No. of ears per plant at maturity	Height of base of ear cm.	Height to apex of ear cm.	Length of ear (by difference) cm.	Weight of single complete plant excluding roots gm.
1. Grain contaminated with spores ( <i>Tilletia tritici</i> ). Bunted plants	62	9.5 ± .215	71.25 ± .886	81.06 ± .952	9.78	24.84 ± .716
2. Grain contaminated with spores ( <i>Tilletia laevis</i> ). Bunted plants	24	9.16 ± .358	79.90 ± 1.03	90.20 ± 1.05	10.30	26.60 ± .914
3. Control	36	8.70 ± .245	81.90 ± .840	92.20 ± .869	9.70	28.70 ± .816
4. Apparently healthy plants from (1). ( <i>T. tritici</i> )	9	8.40 ± .181	87.69 ± 1.426	98.51 ± 1.377	10.82	31.78 ± 1.386
5. Apparently healthy plants from (2). ( <i>T. laevis</i> )	16	9.12 ± .259	82.30 ± 1.30	93.80 ± 1.49	11.50	30.30 ± .972
Treatment	Weight of ears per single plant gm.	Weight of grain per single plant gm.	No. of grain per single plant	Weight of healthy grain per plant gm.	Weight of roots only gm.	Weight of straw only gm.
1. Grain contaminated with spores ( <i>Tilletia tritici</i> ). Bunted plants	10.05	6.15	309	3.85	1.62 ± .055	14.79
2. Grain contaminated with spores ( <i>Tilletia laevis</i> ). Bunted plants	10.63	6.86	251	5.31	2.10 ± .111	15.97
3. Control	13.45	9.16	260	9.16	2.09 ± .105	15.25
4. Apparently healthy plants from (1). ( <i>T. tritici</i> )	14.05	10.03	269	10.03	2.14 ± .136	17.73
5. Apparently healthy plants from (2). ( <i>T. laevis</i> )	14.20	10.02	252	10.02	2.01 ± .098	16.10

When partially-bunted plants are considered, the influence of the fungus is shown in the following average figures obtained by classifying the heads as healthy, semi-bunted and completely-bunted. Where the infection of the head was complete the height was reduced by 15 and 19 per cent. in the two series, but where the infection was partial the height varied from apparently healthy tillers by only 1 and 7 per cent. In these plants, therefore, the height of individual tillers shows the same correlation with the degree of infection as the average height of tillers on plants when these are classified on the same basis with the single plant as the unit.

Table VI.

*Showing the average height of tillers from plants partially bunted.  
Hen Gymro, 1924.*

Series	Healthy ears cm.	Semi-bunted ears cm.	Completely-bunted ears cm.
Small grain	96.7	95.7	82.0
Large „	104.5	97.2	84.4

The weight of straw is clearly a function of the number of tillers at maturity and their height. In these experiments, with the exception of the semi-bunted plants in the large-grain series 1924, the bunted units, in spite of their superiority in the number of tillers fall slightly below the control plants in weight of straw.

As has been stated, measurements on height were made throughout the growing season at weekly intervals. For the first month (1925) no constant difference between the bunted series and the control was obtained, the bunted (*T. tritici*) leading slightly in measurements of the first and third tillers, while the reverse condition held in the case of the second tiller. An indication that bunted plants were falling behind the control in growth appeared in the measurements obtained in May and June, which were as follows:

*Average height in cm. of healthy and bunted plants.*

	May 27th	June 3rd	June 10th	June 17th
Healthy	51	64	79	88
Bunted ( <i>T. tritici</i> )	49	62	78	85

It is obvious that with such a small average difference bunted plants could not be detected by the eye on this character<sup>1</sup>.

While dealing with the retarding influence of the fungus on growth it is interesting to find that in 1925 where data were obtained on the weight of roots, a significant difference amounting to 23 per cent. was shown in favour of the control plants as compared with those infected by *T. tritici*.

Turning now to the question of tillering, the fungus has apparently the reverse effect to that recorded for growth in height. In 1924 with both series of plants the healthy controls showed fewer heads at harvest than plants which were completely bunted, the difference being approxi-

<sup>1</sup> It was necessary to obtain measurements on each plant in the series and re-group them at a later date when the plants could be classified on the presence and absence of bunted grain in the ear.

mately 14 per cent. in each case (Table IV). In 1925 bunted plants (*T. tritici*) again showed increased tillering as compared with the control (Table V). At harvest the differences amounted to only 8.4 per cent. as compared with 14 per cent. with Hen Gymro in the previous experiment, but the difference was more striking when the plants were compared at the period of maximum tiller development. In Fig. 1 the relative tillering of the three series of plants is shown graphically for the complete growing period. In this character the two species of *Tilletia* showed a

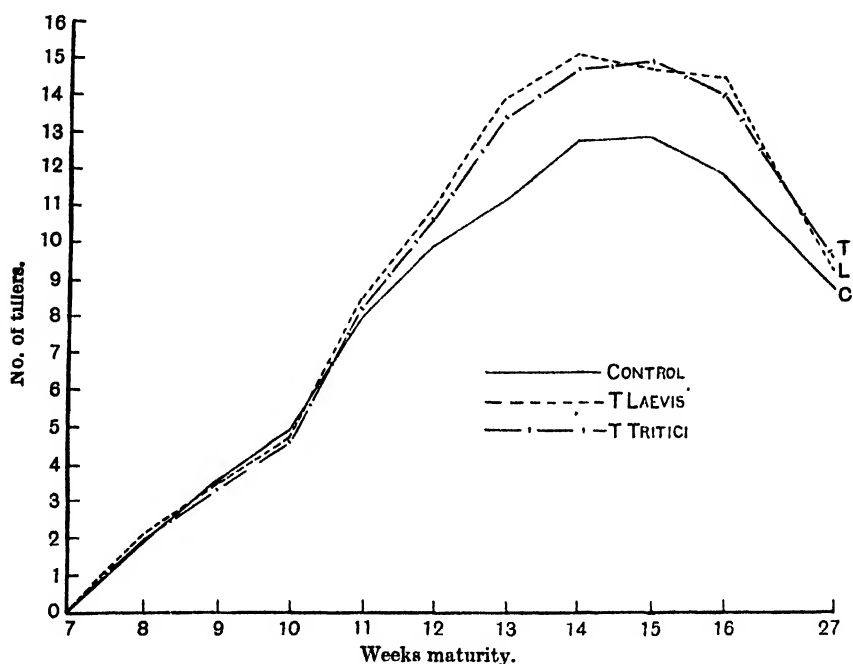


Fig. 1. Graph showing the average number of tillers per plant at weekly intervals. Healthy and bunted plants. Variety: April Bearded. Sown March 4th, 1925.

remarkably close parallel. In the fourteenth week when the maximum number of tillers was recorded the bunted plants of both series showed an increase of 15 and 17 per cent. respectively over the control.

In another experiment in which the plants were grown in rows under field conditions a similar difference in tillering was recorded between bunted and healthy plants, although owing to the influence of competition the actual number of heads produced at harvest was much smaller than in the pot experiments here described. The figures for this experiment are as follows:

Table VII.

*Showing relative tillering of healthy and bunted plants grown in rows under field conditions, 1924.*

Variety	Number of plants examined	Total number of heads at harvest	Average number of heads per plant
Hen Gymro, 24 pure line selections:			
Infected ( <i>T. tritici</i> )	566	2007	3.55
Healthy	269	820	3.05
Twelve other varieties ( <i>T. vulgare</i> ):			
Infected ( <i>T. tritici</i> )	74	233	3.15
Healthy	163	473	2.90

Before leaving the question of growth and yield, reference should be made to the data obtained in this connection from plants derived from bunt-contaminated seed which failed to show any diseased grain in the ear, and were classed, therefore, as "apparently healthy." In both seasons' experiments such plants were found to agree more closely as regards height, length of ears and weight of roots (one season) with the healthy plants in the control series than with the plants obviously bunted. The same is true also in regard to tillering with the exception of the *T. laevis* series in 1925. In these experiments the plants gave in most cases even slightly higher figures than the healthy control plants, suggesting that they had perhaps escaped or grown away from the disease by reason of their extra vigour. The number of such plants which came under investigation is, however, too small to admit of reliable conclusions on this point. In certain experiments conducted in Russia (10) "apparently healthy" plants raised from contaminated seed were found to occupy a position intermediate in regard to height between healthy control and obviously bunted plants.

Except where special reference has been made to the tillering of plants affected by *T. laevis* in the 1925 experiment, the above conclusions refer to the species *T. tritici*. From Table V it is evident that the plants affected by *T. laevis* show in their length of straw, weight of straw and weight of roots, only slight differences when compared with healthy control plants. In regard to height the difference though slight is in the same direction as that shown by the plants infected with *T. tritici*. That the retarding influence of *T. laevis* on growth in height is considerably weaker than that of *T. tritici* has been stated by Potter and Coons (11) in America, and a similar result was obtained in one set of experiments by Mourashkinski (10).

Special attention was paid in these experiments to the possibility of distinguishing bunted and healthy plants in the early growth stages, particularly as it has been stated that infected plants are more luxuriant in growth and darker in colour. The first point appears to be true in so far as the average tillering capacity is concerned, but this feature was not sufficiently marked to make it possible to identify with certainty bunted plants without examination of the ears. The writers failed also to detect any constant variation in the colour-shade of the vegetative organs, but a distinct difference in colour was apparent in the ears, especially towards maturity. Healthy ears changed from green to yellow before those that were diseased, and there came a period when the bunted plants were conspicuous by reason of the dark or blue-green colour of the pales and glumes. At an earlier stage of development the infected plants could only be recognised by opening the sheath and examining the young ovaries. As described by Barrus<sup>(1)</sup> the ovary of bunted plants is then considerably swollen and bright green in colour, the stamens are short and the anthers greatly reduced in size. No exertion of anthers takes place from bunted flowers, a fact which serves as a further distinguishing mark of infected plants when the ear has emerged from the sheath.

The white appearance and the spreading position of the glumes in ripe bunted ears is as familiar as the rounded form of the bunted grains. In some ears affected by *T. laevis* abnormally long grains were found, in some cases stretching as far as 0.5 cm. beyond the pales. On closer examination it was found that the shape of the grain could not be used as a reliable means of distinguishing the two species of *Tilletia* and it was necessary to resort to the microscope and to determine the character of the spore wall.

#### IV. SUMMARY AND CONCLUSIONS.

Summarising the results of the experiments described in the present paper, it is evident that bunt has a distinct influence on vegetative organs of the plant in addition to the well-known effect on the grain. Both species which cause bunt, *Tilletia tritici* and *T. laevis*, were included in one experiment only, from which it appears that the influence of the latter species, while tending in the same direction, was distinctly weaker in its effect than *T. tritici*. This conclusion is in harmony with the results of other workers (4, 10, 11).

The following conclusions refer to *T. tritici*, with which species the work was mainly carried out:

(1) *Soil germination and establishment.*

The actual percentage of germination has not been found to differ appreciably in bunt-free and bunt-contaminated samples, but in one experiment a considerable difference was manifest in the final establishment, the bunt-free samples showing an increase of 25–37 per cent. when compared with those which were contaminated with spores before sowing (Table I). The greatest difference (37 per cent.) was shown by the variety Browick, which gave the lowest establishment figure and had presumably suffered most intensely from the unfavourable climatic conditions.

(2) *Early growth.*

The first visible symptoms of a retarding influence on growth are not always manifest at the same stage of development in the wheat plant. Under one set of conditions (p. 92) the effect was clearly shown by plants in the seedling stage, since those derived from bunt-contaminated seed differed from the control by 14–17 per cent. in height, and 16–20 per cent. in the dry weight of 100 shoots. In another experiment (p. 97) where a considerable reduction in height was evident at maturity the plants showed no appreciable effect during the first ten weeks of growth.

(3) *Tillering.*

In the matter of tillering the influence of the fungus was in the opposite direction to that recorded for growth in height, bunted plants at the period of maximum tiller development producing in one experiment 16 per cent. more tillers than healthy plants grown under precisely the same conditions. At maturity in both cases the number of heads was considerably less than the total number of tillers produced, but bunted plants still showed an increase over those that were healthy (Experiments III and IV).

A similar difference was noted in the case of plants grown in rows in the field<sup>1</sup>.

(4) *Length of straw at maturity.*

A reduction in the length of straw appears to be one of the most constant results of the influence of the fungus on vegetative growth. It will no doubt be shown that the degree of reduction varies with the variety, the season and possibly with the origin of the spores used for contamination. In the experiments here described a reduction in height

<sup>1</sup> Increased tillering and reduced height have been recently described in oat plants affected by loose smut (*Ustilago avenae*) (14).

amounting to 16–19 per cent. in the case of Hen Gymro and 13 per cent. in the case of April Bearded was recorded. It was shown moreover that the reduction was considerably greater in the case of tillers carrying heads in which every grain was bunted than in the case of those bearing both healthy and bunted grain.

(5) *Length of ripe ear.*

In the case of Hen Gymro wheat the fungus showed a slight retarding effect on the length of the ear, while in the variety April Bearded the bunted ears were, if anything, slightly longer than the normal. More data are desirable on this point, since the influence on this organ is probably largely determined by the morphology of the ear in any particular variety. It appears to be generally recognised that ears of the *compactum* type are abnormally narrow and long when bearing bunted grain (6, 15). Plate VII shows bunted and normal ears of *T. vulgare* var. Standard Red, which possesses normally relatively wide, dense ears. For comparison are shown healthy and diseased heads of Hen Gymro, which has ears characteristically lax.

(6) *Root development.*

In one experiment where data were obtained on the dry weight per 100 plants of the roots at maturity a significant difference was apparent between bunted and healthy specimens, the former showing a decrease of 22 per cent.

From the practical point of view, where the final yield of healthy grain is of primary consideration, the most important aspect of the question, apart from the effect on the grain itself, is probably the influence of the disease on establishment and early growth. It is of course recognised that any check which the plant receives in the early stages of growth will be exaggerated later in the case of plants placed in competition with healthy individuals, as must happen in a partially bunted field crop. The full extent of the retarding influence on growth was possibly not exhibited in certain of the experiments under review since the bunted plants were not seriously subject to competition.

Since it has been shown that the presence of the parasite tends to increase tillering, the reduction in heads at harvest which was a striking feature of previous treatment experiments must be attributed either to the stimulating effect of the chemical used for treated lots, or to the depressing influence of bunt on establishment in the field. From the data here given the second alternative seems the more feasible, but since the influence of bunt on establishment is, perhaps more than any other,

likely to be affected by seasonal conditions and to differ with the variety and the origin of the seed, it is desirable to obtain further data on this aspect of the problem, using a large number of seed samples and varieties and a wide range of environmental conditions.

#### V. ACKNOWLEDGMENTS.

The authors desire to express their most cordial thanks to Professor R. G. Stapledon for placing at their disposal the facilities of the Welsh Plant Breeding Station, and for his helpful interest in the work. They desire also to thank Mr J. W. Watkins for his careful supervision of cultural details. The authors are indebted also to Dr E. J. Butler and to Dr G. H. Pethybridge for references to papers bearing on the subject of this paper.

#### VI. LITERATURE CITED.

- (1) BARRUS, F. M. (1916). Observations on the pathological morphology of stinking smut of wheat. *Phytopath.* vi, 21-28.
- (2) EDLER, W. (1903). Einwirkung des Frostes auf den Squareheadweizen. *Illust. Landw. Zeit.* No. 60.
- (3) FARIS, JAMES A. (1924). Factors influencing the infection of wheat by *Tilletia Tritici* and *T. laevis*. *Mycologia*, xvi, 259-282.
- (4) HARWOOD, P. M.. Smut in oat and wheat; Jensen or hot water treatment. *Mich. Agric. Exp. Stat. Bull.* LXXXVII, 10 pp. Ed. 3. 1892 by P. M. Harwood and P. E. Holden. 1892 quoted from (11).
- (5) HEALD, F. D. (1921). The relation of spore load to the percentage of stinking smut appearing in the crop. *Phytopath.* xi, 269-278.
- (6) HEALD, F. D. and WOOLMAN, H. M. (1915). Bunt or stinking smut of wheat. *Wash. Agric. Exp. Stat. Bull.* CXXVI, 24 pp., 5 figs.
- (7) HEGGI, D. (1911). *Étude sur les Ustilaginées*. I. La carie du blé. Magyarovar.
- (8) LANG, W. (1917). Über die Beeinflussung der Wirtspflanze durch *Tilletia Tritici*. *Zeitschr. für Pflanzenkrankh.* xxvii, 80.
- (9) MICZYNSKI, K. (1911). Der Einfluss des Steinbrandes auf die Form der Weizenähren. *Zeitschr. Landw. Versuch. in Oesterreich*, pp. 232-235.
- (10) MOURASHKINSKI, K. E. (1925). The influence of bunt on the vegetation of wheat. Reprint from *Trans. Siberian Acad. of Agric.* iv, 15 pp., 4 graphs.
- (11) POTTER, A. A. and COONS, G. W. (1918). Differences between the species of *Tilletia* on wheat. *Phytopath.* viii, 106-113.
- (12) SAMPSON, KATHLEEN and DAVIES, D. WALTERS (1926). Some experiments on the control of bunt in wheat by copper carbonate and other experiments, including data on the growth and yield of treated and untreated grain. *Welsh Journ. Agric.* ii, 188-212.
- (13) SPERLING, E. (1912). Der Einfluss des Steinbrandes auf die Form des Weizenähren. *Illust. Landw. Zeit.* xxxii, 865.
- (14) TALIEFF, V. I. and GRIGOROVITCH, A. I. (1923). On the influence of smut on the host plant. *Trans. Myc. and Phytopath. Sec. Russian Bot. Soc.* i. *Trans. Moscow Branch*, pp. 47-53. *Rev. App. Myc.* iii, 204.
- (15) WOOLMAN, H. M. and HUMPHREY, H. B. (1924). Studies in the physiology and control of bunt, or stinking smut of wheat. *U.S. Dept. Agric. Bull.* No. 1239, pp. 2-29. Washington.
- (16) ——— (1924). Summary of literature on bunt or stinking smut of wheat. *U.S. Dept. Agric. Bull.* No. 1210, pp. 1-44.



## EXPLANATION OF PLATES VI AND VII

## PLATE VI.

Seedlings raised from bunt-contaminated and bunt-free grain of the same variety—*Marshall Foch*—sown November 17th, 1925. The bunted sample is on the left in each case.

- (1) Heated glass-house, seedlings 8 weeks old, partially sterilised soil.
- (2) Cold glass-house, seedlings 16 weeks old, partially sterilised soil.
- (3) Cold glass-house, seedlings 16 weeks old, non-sterilised soil.
- (4) Open cage, seedlings 16 weeks old, non-sterilised soil.

A distinct difference in height was shown in both types of soil between the bunted and healthy plants raised in the cold house (2) and (3).

## PLATE VII.

- (1) Bunted and healthy ears of *T. vulgare*, variety *Standard Red*.
- (2) Bunted (*B*), healthy (*H*) plants of *T. vulgare*, variety *Hen Gymro* derived from contaminated grain.
- (3) Healthy and bunted ears of *T. vulgare*, variety *Hen Gymro*.

*(Received August 9th, 1926.)*

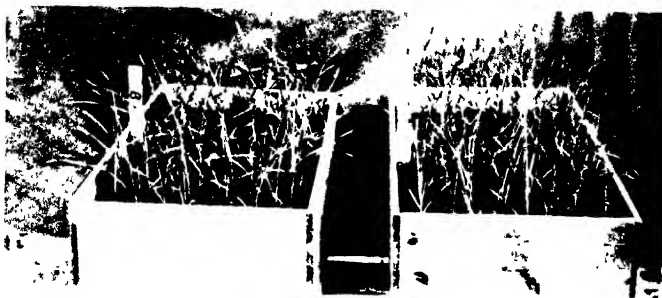


Fig. 2.



Fig. 3



Fig. 4.



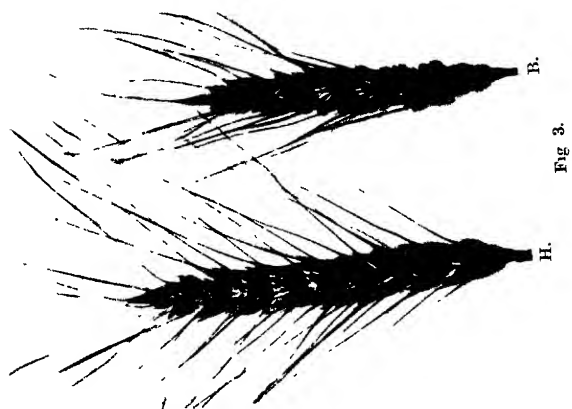


Fig 3.



Fig. 2.



Fig 1.



THE INCIDENCE AND INTENSITY OF *PUCCINIA GLUMARUM* ERIKS. AND HENN., ON WHEAT INFECTED AND NON-INFECTED WITH *TILLETIA TRITICI* WINTER, SHOWING AN APPARENT RELATIONSHIP BETWEEN THE SUSCEPTIBILITY OF WHEAT PLANTS TO YELLOW RUST AND TO BUNT

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(School of Agriculture, Cambridge.)

(With 1 Diagram.)

It is the custom on the University Farm, Cambridge, to carry out various experiments on the control of the bunt fungus, *Tilletia tritici*. The following note records an interesting relationship, observed during the past season 1925-6, between yellow rust of wheat, *Puccinia glumarum*, and bunt, or stinking smut of wheat, *Tilletia tritici*. The wheat in the main block of experiments was sown in October 1925. In May it was observed that certain plots were more yellow than others, and that the yellowing was largely due to a severe attack of yellow rust. Later, when it was possible to identify the plants that were infected with bunt, the plots were more critically examined and it was seen that the bunted plants were all very badly rusted. Since the variety of wheat on which detailed observations were kept was Little Joss, this correlation between bunt and yellow rust was interesting. Little Joss wheat is a cross between Square Head's Master which is neither markedly resistant nor markedly susceptible to yellow rust, and a Russian wheat, Ghirka, which is only slightly susceptible. The degree of rust resistance of this hybrid, Little Joss, compares with the rust resistance of the Ghirka parent.

It is often noticed, however, that in a year when yellow rust is severe Little Joss wheat becomes infected quite early in the season; later, as the plants develop, they "grow away" from the disease and when the plants are in ear only an occasional pustule is found upon the leaves and this generally does not break through the epidermis.

Supporting the evidence of this apparent relationship between bunted and rusted plants there is an isolated observation by Armstrong<sup>(1)</sup> which,

of direct value, confirms the conclusions which have been drawn from the experiments. The following quotations are selected from that paper:

Page 87: "The two extracted rust-resistant types and American Club continued to show very high resistance to attack, but in a few cases this resistance had apparently been more or less broken down. These cases will be noticed after first comparing the number of such apparent "breakdowns" on the different beds."

Page 88: "On bed B (row 2), 1 plant was slightly flecked, and another bore a few unbroken pustules on one blade. In row 10 the 4 plants remained free from infection. In row 6 there were only 2 plants; one of these had 4 leaves badly attacked, and on these over 200 pustules were counted, many of which were freely shedding spores. Some weeks later it was discovered that this plant was also infected with bunt (*Tilletia caries*)."

On the same page there appears the following as a footnote: "Since then another similar case has been observed. In an  $F_2$  culture (from the cross Wilhelmina and American Club) raised from an immune  $F_2$  plant all except one plant were rust-free. This odd plant was very severely attacked by rust, and was also found to be infected with bunt."

In one series of experiments wheat had been sown weekly from October 1925 to March 1926, each plot being of six rows, a row being 16 ft. This wheat had been contaminated prior to sowing with "bunt balls" obtained from Little Joss wheat grown in 1925. It was contaminated at the rate of 1 part by weight of "bunt balls" to 25 parts of weight of wheat; from this stock the requisite amount of wheat was weighed out and sown weekly. On July 1st 500 leaves were picked at random from each of these 24 plots. These leaves were examined in the laboratory for the presence of rust and recorded as having none, slight, moderate or severe. A leaf was recorded as severely rusted if the whole or  $\frac{3}{4}$  of its surface was completely yellow with rust pustules, moderate if  $\frac{1}{4}$  to below  $\frac{3}{4}$  rusted, slight if from a few scattered pustules or streaks to below  $\frac{1}{4}$  rusted. In such a crude classification the main difficulty is in determining whether a leaf is, or is not attacked, since with this variety there are peculiar fleckings on the leaf, and, on these, pustules of yellow rust may, or may not be present. Since the proportion of slight to none, on this account, was thought to be of little value as an indication of intensity of attack, the number of leaves showing a moderate attack was added to the number showing a severe attack. The total, expressed as a percentage of the whole sample (500 leaves) was taken, in what follows, as the "Intensity of yellow rust attack." Table I shows

the values of intensity of rust attack for the series of weekly sowings of bunt-infected "seeds."

At a later date the percentage of bunted ears in these plots was

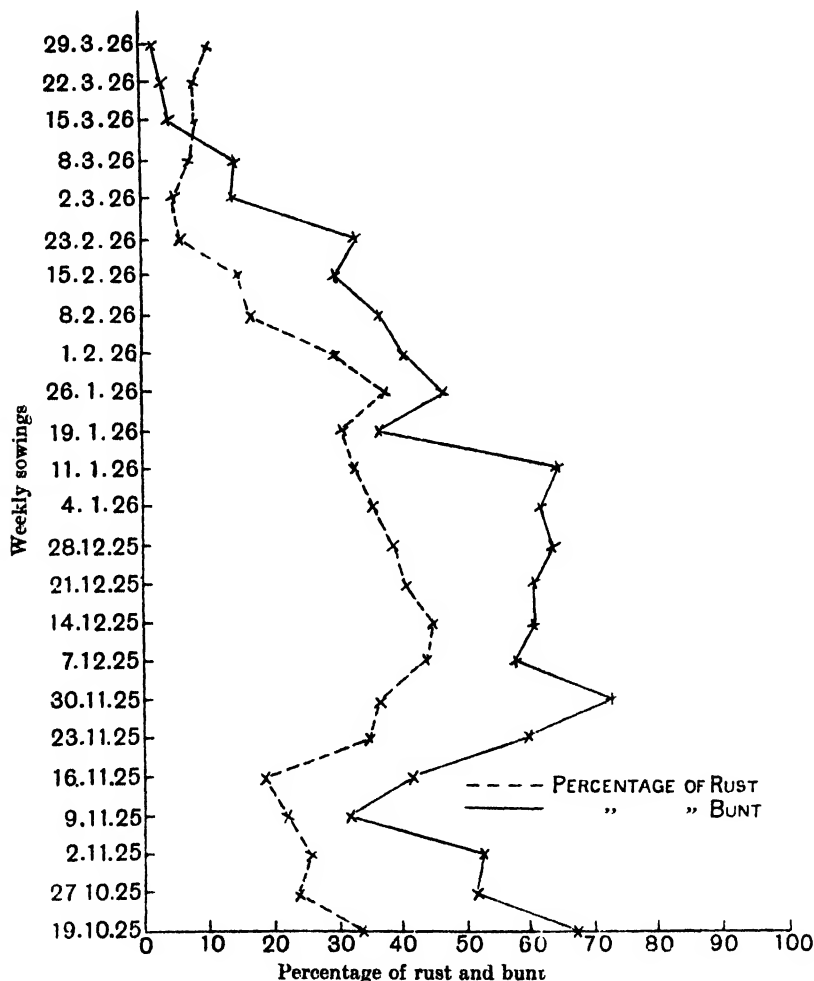


Diagram I. Showing a correlation between the intensity of a yellow rust attack and the percentage of bunt in experimental plots of wheat artificially contaminated at a uniform rate (1.25) with bunt balls and sown weekly from 19. 10. 25 to 29. 3. 26.

estimated by taking a count of 1000 ears from a diagonal band across every plot.

In Diagram 1 the percentage of bunt and the percentage of rust are plotted against the date of sowing. The general trend of values suggests



Table I.

*Showing the intensity of yellow rust attack, and the percentage of bunt, on Little Joss wheat sown weekly from October 19th, 1925 to March 29th, 1926.*

Date of sowing	Intensity of yellow rust attack	Percentage of bunt	Date of sowing	Intensity of yellow rust attack	Percentage of bunt
19. x. 25	33.6	68.2	11. i. 26	32.8	65.2
27. x. 25	23.6	51.9	19. i. 26	31.2	37.4
2. xi. 25	26.4	52.8	26. i. 26	38.4	47.1
9. xi. 25	22	31.8	1. ii. 26	30.2	40.8
16. xi. 25	18.8	41.6	8. ii. 26	17	36.8
23. xi. 25	34.8	60.3	15. ii. 26	15.4	30.4
30. xi. 25	37.3	71.5	23. ii. 26	6	32.9
7. xii. 25	44	58.1	2. iii. 26	5	14.0
14. xii. 25	44.6	61.3	8. iii. 26	7	15.4
21. xii. 25	40.6	60.5	15. iii. 26	7.8	3.9
28. xii. 25	38.8	63.6	22. iii. 26	8.4	3.1
4. i. 26	35.6	61.5	29. iii. 26	9.8	1.0

a definite association between the incidence of rust and bunt in this experiment. It may be, however, that this connection between bunt and rust is spurious. This is to say, it can be argued that, since this wheat was sown on 24 different dates, it was offered 24 different environments, and that these were variably favourable to *both* the diseases and *that* is the true reason why the relationship between their incidence shows up. From the evidence in Tables II and III this is unlikely since in Table II, Plot 1, the percentage of bunted ears is 90 and the lowest percentage of rust is 72. On Plot 4 in a count of 1000 ears there was no bunt and the highest percentage of rust is 2.6. Now Plot 4 was sown within an hour of Plot 1 on the same day. Consider Plots V to XII; these also were sown within a short time of each other on the same day, yet as the percentage of bunted ears increases with the heavier contamination of "bunt balls," so does the percentage of yellow rust increase; and, as the copper carbonate treatment reduces the percentage of bunted ears, so is the percentage of yellow rust reduced. It is a logical conclusion to assume a definite correlation between the two diseases.

In a second series of experiments, and these were the most convincing, Little Joss wheat was contaminated with different amounts of "bunt balls" and sown on the same date. The percentage of bunt and the intensity of rust attack were estimated in the way already described, but where 100 leaves only were examined the uppermost leaf of the stem was observed. These estimates are recorded in Table II. They show

Table II.

*Showing a relationship between intensity of yellow rust and the percentage of bunt in plots of Little Joss wheat which have been contaminated at varying rates with "bunt balls."*

Plot number and treatment	Date of observation	Total No. of leaves examined	Percentage of yellow rust						Percentage of bunt
			None	Slight	Moderate	Severe	None + Slight	Moderate + Severe	
I									
Untreated (1 part by weight of bunt balls to 25 parts by weight of wheat)	20. vi. 26	100	11	17	—	—	28	72	90
	24. vi. 26	1993	8.2	6	—	—	14.2	85.8	
	13. vii. 26	100	10	8	13	69	18	82	
II									
Copper carbonate dusted 3 oz. to the bushel. Contamination 1 : 25	20. vi. 26	100	75	25	0	0	100	0	10.2
	13. vii. 26	100	51	28	5	16	79	21	
III									
Steeped copper sulphate 2½ % Contamination 1 : 25	20. vi. 26	100	56	43	1	0	99	1	2.5
	13. vii. 26	100	74	25	1	0	99	1	
IV									
Steeped formaldehyde 1 : 210. Contamination 1 : 25	20. vi. 26	100	55	41	1	0	99	1	0
	24. vi. 26	1554	92.3	5.1	—	—	97.4	2.6	
	13. vii. 26	100	84	16	0	0	100	0	
V									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 25	14. vii. 26	100	43	36	1	20	79	21	11.5
VI									
Untreated. Contamination 1 : 25	20. vi. 26	100	17	10	—	—	27	73	94
	24. vi. 26	512	9.9	1.8	—	—	11.7	88.3	
	5. vii. 26	500	10.2	13.8	33.8	42.2	24	76	
VII									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 50	14. vii. 26	100	45	49	3	3	94	6	5
VIII									
Untreated. Contamination 1 : 50	20. vi. 26	100	24	17	—	—	41	59	78
	24. vi. 26	185	21	7	—	—	28	72	
	5. vii. 26	500	22	12.8	25.2	40	34.8	65.2	
	14. vii. 26	100	18	30	7	45	48	52	
IX									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 100	14. vii. 26	100	52	17	0	1	99	1	3
X									
Untreated. Contamination 1 : 100	20. vi. 26	100	36	33	—	—	69	31	49.7
	24. vi. 26	360	30.2	9.5	—	—	39.7	60.3	
	14. vii. 26	100	45	25	25	5	70	30	
	5. vii. 26	500	31.2	25	25.2	18.6	56.2	43.8	
XI									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 500	14. vii. 26	100	22	69	7	2	91	9	0.9
XII									
Untreated. Contamination 1 : 500	20. vi. 26	100	73	19	—	—	92	8	25
	24. vi. 26	377	52.8	11.7	—	—	64.5	35.5	
	5. vii. 26	499	43.1	45.1	9.2	2.6	88.2	12.8	
	14. vii. 26	100	44	21	8	27	65	35	
XIII									
Untreated. Contamination 1 : 25	13. vii. 26	100	7	10	15	68	17	83	92.5
LVIII									
A clean sample of seed (?) sown in non-infected (?) ground	24. vi. 26	216	60.6	36.1	—	—	96.7	3.3	1
	8. vii. 26	499	19.6	66.5	12.7	1.2	86.1	13.9	
	13. vii. 26	100	47	52	1	0	99	1	
XXXII									
Untreated. Contamination 1 : 25	8. vii. 26	500	13	37.4	31.4	18.2	50.4	49.6	86.2
	10. vii. 26	500	13.8	26.8	34.8	24.6	40.6	59.4	

Table III.

*Showing intensity of yellow rust on varieties of wheat infected and non-infected with bunt.*

Variety	A Yellow rust intensity in 100 bunted tillers	B Yellow rust intensity in 100 tillers free from bunt
Wilhelmina ... .	100	2
Rector ... ..	100	35
Benefactor ... ..	98	48
Square Head's Master	100	10
Victor... ..	98	6
Marshal Foch ...	100	8
Iron ... ..	100	0
Yeoman ... ..	90	22
Little Joss ... ..	100	0
Rivet* ... ..	90	0
Red Marvel ... ..	93	35

\* Intensity slight, not strictly comparable with intensity in the other varieties.

Table IV.

*Showing the intensity of rust attack on the first four leaves of each of 100 tillers of Rivet wheat, 57 having bunted ears, the remainder being free from bunt.*

Intensity of rust attack	Uppermost leaf	Second leaf	Third leaf	Fourth leaf
None	40	40	36	17
Slight	10	12	9	11
Moderate	48	46	55	68
Severe	2	2	1	4

a relationship between the intensity of yellow rust and the percentage of bunt in plots of Little Joss wheat which has been treated in various ways and has previously been contaminated at varying rates with "bunt balls." In the last series to be described in this note various English varieties of wheat were contaminated at the rate of 1 part by weight of "bunt balls" to 25 parts by weight of wheat, sown, and observed for bunt and rust.

These observations are in Table III; they show the intensity of yellow rust on wheat plants, affected and non-affected with bunt. In recording the observations tillers were taken at random throughout the plot; if the tiller had a bunted ear it was recorded in column A, if it was free from bunt it was recorded in column B. Since this was a field examination, in estimating the intensity of rust, the uppermost leaf was examined, and if showing a slight (+), moderate or severe attack was described as

rusted. Rivet wheat appeared the least susceptible to yellow rust. Previous to the above observations on Rivet wheat this same variety was examined on July 10th. One hundred tillers at random were uprooted and examined leaf by leaf and the intensity of rust recorded. These estimates are in Table IV; they show the intensity of rust attack on the first four leaves of each of the 100 tillers of Rivet wheat, 57 of which had bunted ears, the remainder being free from bunt.

In Table III there is a further possibility of spurious relationship, for it is possible that the tillers which were both rusted and bunted were the very late ones, and it is possible that mere lateness made them prone to both diseases. From the above evidence and from general eye impressions on badly and slightly bunted plots it appeared to the writer that this was not the true explanation, but that in the season 1925-6 there was a very close correlation between yellow rust and bunt, inasmuch as bunted plants were definitely more rusted than plants free from bunt; and, in the case under consideration, it was evident that infection of Little Joss wheat with the bunt fungus was, in effect, capable of breaking down the resistance that Little Joss has, in its later stages, to *Puccinia glumarum*.

The authors (2) of Research Monograph No. 4, Ministry of Agriculture and Fisheries, describe how the  $F_3$  and  $F_4$  cultures of the cross between Wilhelmina and American Club were made use of to test the possibility of breaking down the resistance to yellow rust by manuring excessively with soluble nitrogenous manures. They describe how various "doses" were given to the cultures, the maximum corresponding to the rate of some eight hundredweight of nitrate of soda per acre. They state that, "though the season was unfavourable for rust, and comparatively little was to be found on the other wheat plots, the susceptible cultures were severely attacked whilst those raised from resistant  $F_2$  plants, though examined leaf by leaf from the late spring until the foliage began to die off, remained free, with the exception of an occasional pustule which did not break through the epidermis." On the evidence that is produced below it appears to the writer that rust resistance which cannot be broken down artificially by the use of soluble nitrogenous manures may be broken down by natural contamination of seed wheat with the bunt fungus. It is suggested that Little Joss, to be completely resistant to yellow rust, would need to be resistant to certain other fungoid diseases of which *Tilletia tritici* is one. The converse, however, that a wheat variety immune to bunt must be immune to yellow rust does not follow, since wheat varieties resistant to bunt that we have had under observation for two years have been badly rusted.

## SUMMARY.

In the season 1925-6 an interesting relationship was noticed between yellow rust of wheat, *Puccinia glumarum* and bunt or stinking smut of wheat, *Tilletia tritici*; it was observed that:

1. Bunted Little Joss wheat plants were badly rusted and that plants free from bunt were free or comparatively free from rust.

2. Bunted plants of other wheat varieties were definitely more rusted than plants free from bunt.

3. It is suggested that rust resistance which cannot be broken down artificially may be broken down by natural contamination of wheat with the bunt fungus.

## LITERATURE.

- (1) ARMSTRONG, S. F. (1922). Mendelian inheritance and yellow rust in wheat. *Journ. Agric. Sci.* XII.
- (2) BIFFEN, R. H. and ENGLENDOW, F. L. (1926). Wheat Breeding Investigations at the Plant Breeding Institute, Cambridge. *Research Monograph*, No. 4. Ministry of Agriculture and Fisheries.

(Received August 21st, 1926.)

# OBSERVATIONS ON THE INSECT CARRIERS OF MOSAIC DISEASE OF THE POTATO.

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(With Plates VIII-X and 1 Text-figure.)

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## 1. INTRODUCTION.

THE observations recorded in the following pages are from experiments upon mosaic disease of potatoes carried out during the three or four years previous to the end of 1925 and deal with the part played by insects in its dissemination.

The first and most important step was definitely to ascertain which, if any, of the insect fauna of the potato plant were instrumental in transmitting this virus disease, and this was the object of the experiments here detailed; such points as the optimum condition of plant and insect for infection, the actual organ or organs in the body of the insect which contain the virus and the possibility of inherited infectivity in the insect, etc., are left for future study.

The writer is indebted to Dr George H. Pethybridge for kindly criticising the ms. Acknowledgments are also due to Dr James Davidson for suggestions regarding the insect-proof cages, to Mr H. Britten for taking some of the photographs, to Dr T. Whitehead for supplying some of the mosaic-infected potato tubers, and to Mr Theobald and Dr C. L. Walton for supplying some of the aphides.

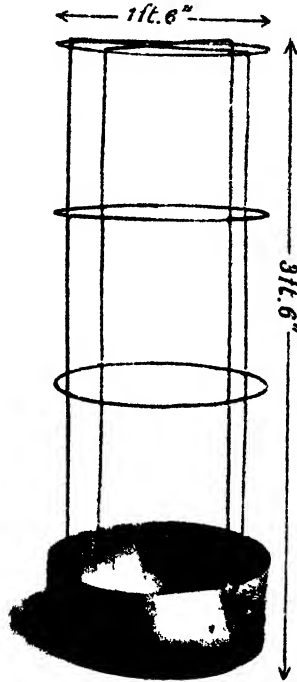
## 2. TECHNIQUE.

In view of the great difficulty experienced by workers on virus diseases of the potato in obtaining an absolutely insect-proof, and especially aphid-proof frame, it may not be out of place to describe the various types of cage used in these experiments prior to the adoption of the frame now in successful use.

Two types of wooden frame covered with fine quality muslin or twisted linen thread were first used, the earlier type made of inch square laths, 2½ ft. high by 4 ft. long. This was soon discarded for the second type of wooden frame which was 4 ft. high by 6 ft. long and was divided into three compartments, each compartment holding one plant, screened from its neighbour by a canvas partition. A small trap door gave access to the plant. The bottom of the frame was provided with a strong tarred base which enabled the whole structure to be sunk in the ground to a depth of 6 in.; the frame was covered with a strong linen net. Neither of these wooden frames proved very efficient, aphides gaining entrance in a number of cases. Probably the ill-success of these frames was due more to the failure of the material covering them, than to any defects in the construction of the frame itself. Many kinds of material, both metal and fabric were tried, but all succumbed to the unusual acidity of the Manchester atmosphere and it was not until the frame, now in use, was evolved that a really aphid-proof structure could be found. The next attempt at an insect-proof cage consisted of a large glass cylinder covered with a canvas top which was placed over the growing plant. This proved to be effective so far as excluding aphides was concerned, but the conditions prevailing inside the cylinder were such as to "draw" the plant unduly. These cylinders proved however to be of the greatest use for breeding upon infected potato plants the insects, which were later to be used as carriers of the virus.

Finally, a cage which was insect-proof and which yet allowed the plant to grow fairly normally, was obtained. This is a modification of one used by Davidson in his aphid work at Rothamsted, and is illustrated in Text-fig. 1. It consists of a circular framework made of brass wire, 18 in. in diameter and 3 ft. 6 in. high. The base of the frame is fitted with a cylinder of heavy galvanised iron, which allows the structure to be sunk to a depth of 6 in. in the soil. Over the frame is fitted a cylindrical covering made of strong unbleached calico, and this is doubly bound with tarred twine round the top of the iron base to prevent slipping. It was found necessary to use brass wire for the frame-

work, as iron wire, even if tinned over, rusted through the canvas. This cage was used successfully for the transmission experiments in 1925. In 1926 it was further improved and will be described in the account of



Text-fig. 1. The brass framework of the cages used in the 1925 experiments 3 ft. 6 in in height and 1 ft. 6 in in diameter. The cylindrical iron base which is sunk into the ground is shown

that year's experiments. One disadvantage of this cage is that it has to be lifted bodily to gain access to the plant within, thus exposing the latter to the danger of chance infection by flying aphides. but with a little practice this can be avoided.

### 3. DESCRIPTION OF EXPERIMENTS.

The inoculations described in these preliminary experiments were of two kinds; that is, efforts were made to induce the various insects to inoculate a potato plant with mosaic disease (*a*) by feeding on the haulm.



(b) by feeding upon the sprouts of the tuber. In both cases the insects used were infected, or presumably infected, with the mosaic virus by being bred upon a "mosaic" potato plant inside one of the glass cylinders previously described.

It may be worth while briefly to outline the experiments prior to the year 1925, which, although negative in their results, were yet carefully carried out and should possess some value.

*Experiments in years 1922-4.*

(a) Attempted transmission of mosaic by feeding infected insects upon the haulms of known healthy potatoes. In these experiments six of the large wooden frames were used and two single frames, and lettered *A* to *H*. The potatoes used were Great Scot and the source of infection was a number of Golden Wonder plants affected with mosaic in a mild form. The insects used were the aphides *Myzus persicae*, *Myzus circumflexus*, and the capsid bug *Lygus pabulinus*.

Frame *A*, with three compartments, contained three plants, each from one quarter of the same tuber of Great Scot, while a fourth plant from the fourth quarter was isolated in a single frame *G*. Infected individuals of *Myzus persicae* were then introduced into compartments 1 and 3 of the frame while the plant in compartment 2 and the plant in frame *G* acted as controls.

Frame *B* was treated similarly, except that *Lygus pabulinus* was substituted for the aphid, and the plant from the fourth quarter was isolated in a single frame *H*. The other four frames *C*, *D*, *E*, *F*, each with three compartments were treated in much the same way, using *M. circumflexus* and *M. persicae*. In these, however, the tubers were cut into three parts, the plants arising from each one-third acting as controls to the two experimental plants from the two other thirds respectively.

The results of this experiment, so far as it was carried, were entirely negative, both controls and experimental plants remaining healthy throughout the season. Owing to illness the writer was unable to save the tubers from these plants and grow them the following year; had this been done it is possible that some disease might have made itself evident.

(b) The following sprout inoculations with aphides were also carried out in the same year as the above:

(1) On May 11th, 1922, a number of adult females of *Macrosiphum gei* (= *solanifolii*) were transferred to the sprouts of six half-tubers of healthy Great Scot and placed in aphid-proof glass vessels. These aphides

had been breeding for 21 days upon a plant of Great Scot which was affected with mosaic disease.

A similar number of adult females of *M. gei* from a known healthy Great Scot plant were transferred to the sprouts of the six remaining half-tubers of the healthy Great Scot and placed also in glass containers; these six halves acted as controls to the preceding ones.

(2) The above experiment was repeated, using however another species of aphid (*Myzus circumflexus*). The source of infection this time was a mosaic Golden Wonder, and the six control half-tubers were not fed upon by any aphides from a healthy plant. On May 24th, thirteen days later, the half-tubers were cleared of aphides and planted. The twenty-four plants resulting proved healthy and remained so throughout the summer.

#### *Experiments in 1923.*

In 1923 another short series of sprout inoculations by means of aphides was carried out, this time using the species *Myzus persicae* and *Macrosiphum gei*. The following is a brief description of these inoculations.

(a) On May 18th, twelve adults of *M. gei*, which had been feeding for fourteen days upon a mosaic Great Scot, were placed six each upon two halves of Rhoderick Dhu tubers, the other two halves, untouched by insects, acting as controls. On May 23rd the aphides were removed and the four half-tubers planted.

(b) On May 16th, twelve individuals of *M. gei*, from a mosaic Golden Wonder, were placed upon the sprouts of two half-tubers of Rhoderick Dhu, the other two halves acting as controls. The times of planting, etc., were as in (a).

(c) The same as (b) but using *Myzus persicae* in the place of *Macrosiphum gei* and substituting Great Scot for Rhoderick Dhu.

(d) In this experiment *Myzus persicae* from infected Great Scot were transferred to half-tubers of Edzell Blue. One half-tuber only used in this experiment.

(e) *M. persicae* from mosaic Golden Wonder to half-tubers of Arran Chief. Times and numbers the same as (a), (b) and (c).

#### *Results of 1923 sprout inoculations.*

All the plants resulting from the half-tubers, both experimental and control, remained healthy throughout 1923. The following point of interest arises: in the experiment (d), it was noticed that the source of

mosaic infection, viz. the mosaic Great Scot (used in (d) only) upon which *M. persicae* had been fed, was also showing signs of leaf-roll. Both plants, however, arising from the half-tubers—the inoculated and the control—which were grown under cover remained healthy, but on growing the progeny of these two plants the following year, out of a total of twelve tubers in (d) all gave leaf-roll plants without mosaic, and of the ten tubers of (d<sub>1</sub>) (the control) all were healthy. Conclusions cannot be based upon this isolated occurrence, but the suggestion might be made that *M. persicae* had transmitted leaf-roll to the exclusion of mosaic.

On April 25th, 1924, the tubers resulting from the 1923 sprout inoculations were planted with the following results:

(a) Six experimental tubers gave all healthy plants; seven control tubers gave all healthy plants.

(b) Five experimental tubers gave all healthy plants; five control tubers gave all healthy plants.

(c) Six experimental tubers gave all healthy plants; four control tubers gave all healthy plants.

(d) Twelve experimental tubers gave leaf-roll plants; ten control tubers gave healthy plants.

(e) Six experimental tubers gave all healthy plants; three control tubers gave healthy plants.

This concludes the account of the attempts made up to the end of 1924 to induce insects to transmit mosaic disease of the potato. It is clear that the evidence of such transmission is almost entirely negative, and although aphides under certain circumstances may transmit potato mosaic disease, it seems certain that under other circumstances even when transmission might well be expected to occur, it does not do so.

In 1925 the insect transmission work was restarted on a wider scale, with an improved technique in the shape of reliable insect-proof frames (Plate VIII, fig. 1), and extended to include all the usual Hemipterous insect fauna of the potato plant. These insects have already been carefully studied in regard to their methods of feeding<sup>(9)</sup> upon the potato plant, and certain theories have been put forward as to their capabilities as disease carriers deduced from this study of their feeding methods. It is hoped that these theories may be confirmed or disproved by the inoculation studies now to be described.

*Description of 1925 experiments.*

The following insects were used in the 1925 experiments:

## HEMIPTERA.

## HETEROPTERA.

- CAPSIDAE.            *Calocoris bipunctatus*.  
                         *Lygus pabulinus*.

## HOMOPTERA.

- TYPHLOCYBIDAE. *Zygina pallidifrons*.  
                         *Eupteryx auratus*.  
ALEURODIDAE.    *Asterochiton vaporariorum*.  
APHIDIDAE.        *Myzus persicae*.  
                         *Myzus circumflexus*.  
                         *Macrosiphum (solanifolii) gen.*

The work was divided into two parts: (a) the placing of infected insects on the shoots of sprouting half tubers; (b) the placing on the haulms of growing healthy plants of insects previously infected as a result of feeding upon mosaic diseased potato plants. In order to avoid introducing a possible complicating factor, the same variety of potato was used as far as possible for the source of insect infection and for the experimental transmission. This variety was President, the source of infection being plants affected with mild mosaic and the potatoes for infection being selected Scotch seed.

The presumably infective insects for the experiments were raised as follows:

Six mosaic plants were grown in 12-inch pots, each plant confined under a tall glass cylinder fitted with a gauze top. On these six plants the six species of insects used were fed. In every case ample time was allowed for a given species of insect to become infected; in most cases, and especially with aphides, the insects were bred upon the infected potato plant. The capsid bugs were captured in the first and second larval instars and allowed to come to maturity under the glass cylinder upon the mosaic plant.

(a) *Inoculation experiments with insects upon the sprouts of tubers.* These inoculations were entirely negative in their results and as such will be only briefly outlined. A number of half-tubers of President (other halves as control), were placed in glass vessels with canvas tops. On the sprouts of the experimental halves the infected insects under trial were placed, the number of insects introduced on each half-tuber

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varying from six to twelve. It was found that this method of inoculation was extremely unsatisfactory for all the insects except the aphides. The leaf-hoppers and white-fly died without feeding upon the sprouts, and the capsid bugs were not observed to puncture the sprouts.

The insects were placed upon the tubers on June 4th; they were removed on June 16th and the tubers then planted.

All the plants resulting from both experiment and control were healthy with the exception of (a) and (a<sub>1</sub>) (*Myzus persicae*) where leaf-roll disease developed in both experiment and control.

(b) *Inoculation experiments with insects upon the haulm.* These experiments were carried out upon two plots of land at the University experimental grounds, Fallowfield, Manchester. On Plot 1, the larger of the two, twelve selected tubers of President were used. Each of these was divided into two, and each half was grown under an insect-proof cage, thus giving twenty-four cages on this plot. The half-tubers were planted and earthed up, and the cage placed in position at the time of planting to avoid any accidental insect contamination. Prior to planting, the tubers had been kept in an insect-proof receptacle and each tuber was examined before being planted for any signs of sprout-infesting aphides or other insects.

Plot 2 was treated similarly, using Great Scot in the place of President. In this case only twelve cages giving six experimental half-tubers and six controls were used, instead of twenty-four as with President.

A photograph of one of these experimental plots is shown in Plate VIII, fig. 1, the frames being lettered in the case of President, as follows:

### *President.*

APHIDIDAE.	<i>Myzus persicae</i>	...	...	...	G, G <sub>1</sub>
	"	...	...	...	H, H <sub>1</sub>
	<i>M. solanifolii</i>	...	...	...	I, I <sub>1</sub>
	"	...	...	...	J, J <sub>1</sub>
	<i>M. circumflexus</i>	...	...	...	K, K <sub>1</sub>
ALEURODIDAE.	"	...	...	...	L, L <sub>1</sub>
	<i>A. vaporariorum</i>	...	...	...	M, M <sub>1</sub>
	"	...	...	...	N, N <sub>1</sub>
CAPSIDAE.	<i>Calocoris bipunctatus</i>	...	...	...	O, O <sub>1</sub>
	<i>Lygus pabulinus</i>	...	...	...	P, P <sub>1</sub>
TYPHLOCYBIDAE.	<i>Eupteryx auratus</i>	...	...	...	Q, Q <sub>1</sub>
	<i>Zygina pallidifrons</i>	...	...	...	R, R <sub>1</sub>

The second symbol,  $G_1$ ,  $H_1$  etc., indicates the control in each case. It will thus be seen that two tests were made with each of the three species of aphid and with the white-fly, but only one with each of the two species of capsid bug and leaf-hopper. With Great Scot the twelve cages were numbered 1 to 6, and  $1_a$  to  $6_a$ , the latter being the controls, thus giving one test to each species of insect.

*Description of transmission experiments with variety President.*

*Plot I.*

The twenty-four half-tubers were planted and the cages placed in position on May 14th, 1925; and the following details are given concerning each experiment:

*Myzus persicae* (insects from sprouting potato tubers). Twenty-four wingless females were placed on each of the two experimental plants on June 10th, the plants being then 6 to 8 in. high, healthy and free from insects. Four weeks later the two experimental plants were cleared of aphid by spraying. No accidental infection by other insects took place in either experiments or controls, all four plants were inspected at intervals and remained healthy to the end of the season when the plants were dug up and the tubers collected for growing the following year.  $G$  gave six tubers,  $G_1$  gave four tubers,  $H$  gave six tubers,  $H_1$  gave two tubers.

*Macrosiphum (solanifolii) gei*. The insects originally came from a rose. The dates, numbers of insects and general procedure, were the same as in the preceding case. The plants remained apparently healthy throughout the season and no accidental contamination of either experimental or control plants took place. On harvesting,  $I$  produced eight tubers,  $I_1$  produced three tubers,  $J$  produced five tubers and  $J_1$  four tubers.

*Myzus circumflexus* (insects from chrysanthemum). Details of this experiment are as with the two other aphid species.

In view, however, of the results obtained in 1926, after planting out the resulting tubers, the observations on these inoculations during 1925 are given in full, as follows:

- $K$ . June 3rd. Plant 6 in. high apparently healthy, no insects present.  
 „ 10th. *M. circumflexus* introduced. Plant apparently healthy.  
 „ 17th. Traces of mottling visible on the young leaves.  
 „ 24th. July 2nd and July 6th. Mottling still present.  
 July 7th. Plant sprayed.  
 „ 13th. Aphides dead, mottling and puckering present, suggestive of mild mosaic.

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- $K_1$ . June 19th. Plant apparently healthy, no insects present.  
    „ 24th. Slight signs of mottling.  
July 2nd. Mottling still present.  
    „ 6th. Mottling less distinct.  
    „ 13th. Apparently healthy, no insects present.
- $L$ . June 3rd. Plant 6 in. high, no insects present, apparently healthy.  
    „ 10th. *M. circumflexus* introduced.  
    „ 17th. Slight mottling visible on young leaves.  
    „ 24th. Mottling still present.  
July 2nd. Mottling more pronounced.  
    „ 6th. Mottling and puckering developing.  
    „ 7th. Plant sprayed.  
    „ 13th. Aphides dead, mottling still present.
- $L_1$ . June 19th. Plant healthy, no insects present.  
    „ 24th. Some signs of mottling.  
July 2nd. Some symptoms of mosaic.  
    „ 6th. Symptoms increased.  
    „ 13th. Symptoms still persisting. plant free of insects.

On harvesting,  $K$  gave three tubers,  $K_1$  gave five tubers,  $L$  gave seven tubers (very small),  $L_1$  gave three medium-sized tubers.

### *Asterochilton vaporariorum*, white-fly.

Twenty-four individuals of the greenhouse white-fly were placed upon each of the two plants  $M$  and  $N$  on June 10th, the plants being then about 6 in. high, healthy and free from insects. The white-fly continued to feed on these potato plants until July 13th when the plants were sprayed. Both experimental and control plants remained apparently healthy throughout the summer, and on harvesting gave the following numbers of tubers:  $M$  gave four tubers,  $M_1$  gave two tubers.  $N$  gave six tubers,  $N_1$  gave three tubers.

### *Calocoris bipunctatus*, *Lygus pabulinus*, Capsid bugs.

On June 29th, twelve individuals of the two species of capsid bug were placed upon the plants  $O$  and  $P$ . *C. bipunctatus* on  $O$ , and *L. pabulinus* on  $P$ . On July 13th the plants were examined, the capsid bugs were still living and the plants showed a certain amount of damage from the feeding of the bugs, but were otherwise healthy.

Both plants and their controls remained healthy throughout the summer. On harvesting,  $O$  gave three tubers,  $O_1$  gave two tubers,  $P$  gave three tubers,  $P_1$  gave five tubers.

*Eupteryx auratus*, *Zygina pallidifrons*, Leaf-hoppers.

Twelve individuals of *E. auratus* and twelve of *Z. pallidifrons* were placed on June 16th on the two plants *Q* and *R* respectively. The plants were inspected at weekly intervals till July 13th when they were sprayed, leaf-hoppers were still present on that date, and in the case of *Q*, the plant though badly marked by the leaf-hoppers appeared otherwise quite healthy. On June 22nd both *R* and *R*<sub>1</sub> showed unmistakable signs of mosaic infection, these two plants were therefore discarded and this experiment repeated. New half-tubers were planted on June 22nd and the insects placed on the plants on July 10th. Both *R* and *Q* and their controls remained apparently healthy except for the puncture marks of the leaf-hoppers. On harvesting, *Q* gave four tubers. *Q*<sub>1</sub> gave three tubers, *R* gave four tubers. *R*<sub>1</sub> gave four tubers (large).

*Plot II.*

In this plot as already described twelve cages only were used, giving six experimental frames and six controls, the variety Great Scot was substituted for President. The same insects were used as in the foregoing, there being, however, only one inoculation test with each species of insect, viz. one capsid (*C. bipunctatus*) and one leaf-hopper (*Z. pallidifrons*) being used

The results of this experimental plot were entirely negative, both inoculated and control plants remaining healthy throughout the summer. On removing the cages and digging the plants at the end of September. it was found that no tubers had been formed; further observations were therefore impossible.

The tubers resulting from the plants on Plot I, variety President, were all small in size, although sufficient to carry on the plants the following year. A point of some interest arises from a comparison of the tubers produced by the inoculated plants of President with the uninoculated control plants, namely that the progeny of the controls was always superior in size, though not necessarily in number, to the progeny of the experimental plants. This the writer puts down to the deleterious effect of the feeding of the insects introduced under the frames. Such reduction in the size of the tuber was especially marked in plants *O*, *P*, *Q* and *R*, where the insects used, capsid bugs and leaf-hoppers, are particularly injurious to plant foliage.

The tubers were carefully collected, each being provided with its identification mark in Indian ink and stored in silver sand. The progeny of each plant was kept in a separate receptacle.



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Later, the silver sand was discarded and the tubers were stored in canvas-covered dishes.

*Results obtained in 1926 from the progeny of the plants inoculated under cages in 1925.*

The tubers variety President from Plot I were planted in 1926 in a compost of leaf mould and soil contained in 10-inch pots. The pots were placed in a cold greenhouse which was free from aphid, having had no plants in it during the winter. Each pot was labelled and an additional wooden label was fixed in the soil. The following results were obtained:

### APHIDIDAE.

#### *Myzus persicae*.

- G. Six tubers, six plants, all mosaic.
- G<sub>1</sub>. Four tubers, four plants, all healthy.
- H. Six tubers, six plants, all mosaic.
- H<sub>1</sub>. Two tubers, two plants, both healthy.

#### *Macrosiphum gei* (= *solanifolii*).

- I. Eight tubers, eight plants, four healthy and four mosaic.
- I<sub>1</sub>. Three tubers, three plants, all healthy.
- J. Five tubers, five plants, two mosaic, three healthy.
- J<sub>1</sub>. Four tubers, four plants, all healthy.

#### *Myzus circumflexus*.

- K. Three tubers, three plants, all mosaic.
- K<sub>1</sub>. Five tubers, five plants, one mosaic, four healthy.
- L. Seven tubers, seven plants, all mosaic.
- L<sub>1</sub>. Three tubers, three plants, all mosaic.

### ALEURODES.

#### *Asterochiton vaporariorum* (white-fly).

- M. Four tubers, four plants, two mosaic, two healthy.
- M<sub>1</sub>. Two tubers, two plants. Both healthy.
- N. Six tubers, six plants, all healthy.
- N<sub>1</sub>. Three tubers, three plants, all healthy.

### CAPSIDAE, Capsid Bugs.

#### *Calocoris bipunctatus*.

- O. Three tubers, three plants, all healthy.
- O<sub>1</sub>. Two tubers, two plants, both healthy.

*Lygus pabulinus.*

P. Three tubers, three plants, all healthy.

P<sub>1</sub>. Five tubers, five plants, all healthy.

## TYPHLOCYBIDAE, Leaf-hoppers.

*Eupteryx auratus.*

Q. Four tubers, four plants, one mosaic, three healthy.

Q<sub>1</sub>. Three tubers, three plants, all healthy.

*Zygina pallidifrons.*

R. Four tubers, four plants, one mosaic, three healthy.

R<sub>1</sub>. Four tubers, four plants, all healthy.

In Plates VIII-X are shewn photographs of some of the successful inoculations with their controls.

## 4. DISCUSSION OF RESULTS OF 1925 INOCULATIONS WITH INSECTS.

Before considering the results of these inoculation trials, it will be well to review shortly the conclusions arrived at by other workers in this field. As regards Aphides, Murphy is of the opinion that several species are transmitters of this disease, but he finds that under apparently similar conditions inoculation is often not successful. In a recent paper<sup>(5)</sup> he says: "We have at different times successfully conveyed mosaic and leaf-roll infections from tuber to tuber by means of aphides on the sprouts, but on many occasions the results have been mainly or entirely negative. Thus, the percentage of successful infections varied from 100 to 0, and in spite of much work on the subject the factors governing this variation remain undetermined. Three different aphides have been used at various times, these being *Myzus persicae*, *Macrosiphum gei* (= *solanifolii*) and *Myzus pseudosolani*. All have carried infection at times so that the conflicting results do not depend on the sort of aphid used, neither do they appear to be determined by the number of aphides used, the length of their stay on the source of infection or on the plant being infected; by the medium by which the tubers are sprouted, whether air or soil, by the presence or absence of light nor by the temperature."

These experiments were attempts at transmission from the sprouts of infected tubers to sprouts of healthy tubers, and not as was the case in the writer's experiments from infected haulm to healthy haulm. Schultz and Folsom<sup>(8)</sup> report successful inoculation by means of three species of aphides, *Myzus persicae*, *Macrosiphum gei*, and *Aphis*

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*abbreviata* Patch. These authors find that aphides produce the symptoms of virus disease in the same year when caged upon the haulms, though apparently they also find difficulty with aphid inoculations, as they state (8) p. 526) that: "Aphides sometimes do not transmit disease under conditions that apparently are the same as those giving positive results."

In an earlier paper (4) Schultz and Folsom find that plants treated with virulent aphides may appear healthy but produce progeny that are all affected with mosaic, and again in the same Journal (7) they state that: "*Macrosiphum gei* transmitted mosaic to a number of plants with symptoms only in the progeny." These findings are of interest in view of the writer's results with the same species of aphid.

As regards other insect carriers of potato virus opinion appears to be divided. Murphy (4) seems to be of the opinion that various potato insects other than aphides are carriers of leaf-roll, though not necessarily of mosaic. He gives the following insects as carriers: the capsid bug *Calocoris bipunctatus*, and the Jassid *Typhlocyba ulmi*. To the writer's knowledge the last-named Jassid does not attack potatoes, so possibly the species referred to was *Eupteryx auratus*, which somewhat resembles it and is a common potato insect. Murphy gives his conclusions as follows: "While the above results can show hardly more than a suspicion that the potato flea-beetle can act as a carrier of leaf-roll there is little room for doubt that both capsid bugs and jassids act as efficient transmitters of leaf-roll. This is important as showing that there is no exclusive specific relationship between aphides and the actual cause of leaf-roll which is presumably an organism."

In 1920 Schultz and Folsom (6) stated that: "As yet no other insect (than aphides) is known to carry mosaic of potato." Since then, however, this opinion may have been modified.

There seems little doubt that insects other than aphides act as carriers of virus disease in many plants of different orders. The work of Kunkel (2) and Hartzell shows that the leaf-hopper *Cicadula serotata* is a carrier of the virus disease known as "Aster Yellows." Storey (10) has also shown that a species of leaf-hopper transmits a virus disease of maize known as "Streak." In addition various leaf-eating beetles are thought to be carriers of mosaic of other plants.

Turning now to the results of the present preliminary investigations, some definite evidence of successful transmission of potato mosaic by means of certain insects appears to emerge. It will be seen that with most of the insects one or other of the two inoculations with each insect

is apparently successful. The chief point of difference seems to lie in the *amount* of infection produced by the various insects. Thus in the case of the aphid *Myzus persicae* the six tubers harvested from *G* and the six from *H* gave mosaic infected plants in every case. On the other hand, in *Q* and *R* the inoculations with the two leaf-hoppers produced only one infected plant out of four, the white-fly gave two out of four in one case and none at all in the other. The capsid bugs failed to transmit the disease in both cases. The insects thus appear to vary greatly in the degree of their efficiency as disseminators of virus disease. This may be due either to the method of feeding of the insects or to some requirement of the virus. It may also be suggested that plants *Q* and *R* which only gave one infected plant out of four did not receive the same amount of virus as did plants *G* and *H*, or else successful inoculation was only achieved in one or two stems of the plant. Schultz and Folsom<sup>(6)</sup> find that a plant with three stalks healthy and four mosaic may produce three mosaic tubers and two healthy ones. The number of insects used in each experiment may influence the infection in so far as even distribution over the plant is concerned. In the case of the aphid *Myzus persicae*, out of twelve tubers from the 1925 inoculations, twelve mosaic plants were produced in 1926, while the six control plants remained healthy.

With the second aphid, *Macrosiphum gei*, six mosaic plants were produced out of the total of thirteen tubers from the two inoculated plants, the seven control plants remaining healthy.

It is unfortunate that the trials with the third species of aphid *Myzus circumflexus* for 1925 must be disregarded owing to the failure of the controls to remain healthy.

It is manifestly unwise to make definite statements as to the infective power of these insects at this stage in the investigations, nevertheless it is possible to draw some conclusions. It seems fairly clear that *under certain conditions*, the aphides *Myzus persicae* and *Macrosiphum gei* can act as efficient transmitters of potato mosaic disease. In the present state of our knowledge of this subject it is not possible to say what these conditions may be. Unlike Schultz and Folsom the writer has so far been unable to produce symptoms of mosaic disease in a potato plant in the same year as that in which the inoculations were performed by means of aphides placed upon the haulm. The disease has only shown itself in the plants produced by the progeny of the experimental plant in the following year. In the case of inoculation with aphides, however, upon sprouting tubers, it appears possible to

produce current season symptoms. Atanasoff(1), commenting upon the difficulty and uncertainty of aphid inoculation of the haulm, states that most of the difficulties would disappear if the inoculations were made with the sprouting tuber and the experiment thus confined to one season. Although this is probably true of some aphid inoculations, it does not hold good for transmission experiments with other potato insects, such as capsid bugs, leaf-hoppers and flea-beetles, as these will not feed upon the sprouts of tubers or, in the case of the capsids, can only with great difficulty be induced to do so. For these insects the only method of sprout inoculation would seem to be the removal of the salivary glands and insertion of them into the sprout. This is being carried out in the present season (1926).

Apart from this there seems no alternative to the method of two-season haulm infection, and there is no reason why this should not be reliable if proper care be taken in carrying out the experiments.

In considering the inoculations with white-fly (*Asterochiton*) it is interesting to find infection in one case. Although more cannot be said at this early stage of the work, it seems quite probable that the white-fly is a potential carrier of virus disease, more especially as it is an insect which, like the aphides, habitually taps the phloem in its feeding. Both species of capsid bug failed to transmit the disease, thus in some measure bearing out the suggestion of the writer(9) that insects such as these which possess a salivary secretion violently toxic to the plant host are unlikely to be transmitting agents.

With the two leaf-hoppers one plant only with each species became infected. This very slight infection may be due either to the toxic effect of the saliva upon the plant, or to the methods of feeding of these insects which only occasionally tap the phloem. Leaf-hoppers, especially *Eupteryx auratus*, are sometimes severe pests of the potato plant as in the present summer (1926), and it is important that their connection, if any, with the spread of virus diseases of the potato should be established.

Some possibilities in these insect inoculations of the potato plant must be considered. Firstly the effect of the canvas cages upon the normal growth of the plant, and secondly their effect on the symptoms of mosaic disease. In the 1925 experiments it was found that the plants under the cages were drawn up and sappy, and had to be supported. In addition the tubers appeared to be reduced in size as compared with those of plants grown normally. Apart from this the plants grew strongly and were green, luxuriant and apparently healthy enough.

In regard to the second point it is difficult in the present state of our knowledge to say what the effect of the cages and the consequent alteration of light and temperature would be on the development of virus disease. Atanasoff(1) says on this point: "The potato can grow and develop a normal and very luxuriant growth at comparatively low temperatures if only sufficient sunlight is present. Most of the virus diseases of this plant, on the other hand, require much higher temperature for their development, so that their development can be not only retarded but even completely suppressed by low temperatures which are high enough to permit a normal development of the plant."

Atanasoff goes on to say that it is thus possible for a positively infected tuber to produce under low temperature conditions an apparently healthy plant, thus giving a partial or complete suppression of the disease.

Murphy(3) on the other hand, would appear to hold the opposite view, he says: "Field experiments on mosaic were rendered nugatory so far as the greater part of 1921 was concerned, by the very dry and hot weather which had the effect of suppressing the symptoms partially or almost entirely. It was already known from observation and experiment in Canada (as well as in U.S.A.) that this might happen in certain warm and dry localities and seasons. Emphasis was there laid on the temperature, but the appearance of some mosaic symptoms for the first time in plants presumed infected from the beginning, following the first good rain towards the end of July, would indicate that moisture may also be concerned. It was clearly shown in some of the Canadian experiments referred to, that though the symptoms were suppressed in the case of diseased plants in certain places, with the result that their yield was hardly inferior to that of neighbouring healthy plants, yet the disease was still present and transmissible. It reappeared in the diseased seed in the subsequent year, under conditions more favourable to the disease in at least as intensified a form as before its suppression."

Murphy also says: "27° C. is the optimum for the growth of the potato and this exceeds that necessary for the development of the hypothetical mosaic organism. The latter may be concluded from the fact that the disease is most flourishing under cooler conditions."

In regard to the effect of light upon the development of virus disease Schultz and Folsom(7) state: "Shading tended to increase mosaic mottling and decrease leaf-rolling."

In 1925 when the writer's experiments were carried out the summer was exceptionally hot and dry. The plants, however, were kept well

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watered by flooding the bases of the insect-proof frames. It may be noted that in the case of *L* and *L*<sub>1</sub> and the first *R* and *R*<sub>1</sub>, where the tubers were already affected, the mosaic disease developed normally, the rest of the plants remaining green and apparently healthy till the end of the season. From this it would appear that the presence of the canvas cages does not to any great extent affect the development of the disease symptoms.

### SUMMARY.

1. An account is given of some preliminary experiments with insects as transmitters of potato mosaic disease. The various types of insect-proof cage which have been used in these experiments are described.

2. The following insects were used in the inoculations:

#### APHIDIDAE.

*Myzus persicae*.

*Macrosiphum gei* (= *solanifolii*).

*Myzus circumflexus*.

#### ALEURODES, White-fly.

*Asterochiton vaporariorum*.

#### CAPSIDAE.

*Calocoris bipunctatus*.

*Lygus pabulinus*.

#### TYPHLOCYBIDAE, Leaf-hoppers.

*Zygina pallidifrons*.

*Eupteryx auratus*.

3. The aim of the experiments is definitely to identify what insects of those normally attacking the potato are disseminators of virus disease.

4. Infected insects were placed both upon the sprouts of tubers and upon the haulm; the latter is the only satisfactory method for insects other than aphides.

5. Successful transmission of mosaic disease was obtained in 1925 by means of the aphides *Myzus persicae* and *Macrosiphum gei*. Some evidence of infection by means of *Asterochiton vaporariorum* (greenhouse white-fly) and the leaf-hoppers *Zygina pallidifrons* and *Eupteryx auratus* was also obtained; further work with these insects is required before definite conclusions are drawn. The capsid bugs *Lygus pabulinus* and *Calocoris bipunctatus* failed entirely to transmit the disease.



Fig. 1.



Fig. 2.



Fig. 2A.







Fig. 3A.





Fig. 4



Fig. 4A



Fig. 5.



## LITERATURE CITED.

- (1) ATANASOFF, D. (1924). Methods of Studying the Degeneration Diseases of Potato. *Phytopath.* xiv, No. 11, 521-533.
- (2) KUNKEL, L. O. (1926). Incubation Period of Aster Yellows in its Insect Host. Abstract in *Phytopath.* xvi, No. 1.
- (3) MURPHY, P. A. (1921). Some Recent Work on Leaf-Roll and Mosaic. *Report Internat. Potato Confer. Roy. Hort. Soc.*, p. 148.
- (4) — (1923). On the Cause of Rolling in Potato Foliage; and on some further Insect Carriers of the Leaf-Roll Disease. *Sc. Proc. Roy. Dublin Soc.* xvii, No. 20, 163-184.
- (5) MURPHY, P. A. and M'KAY, R. (1925). Investigations of the Leaf-Roll and Mosaic Diseases of the Potato. *Journ. Dept. of Lands and Agriculture, Dublin*, xxv, No. 2.
- (6) SCHULTZ, E. S. and FOLSOM, D. (1920). Transmission of the Mosaic Disease of Irish Potatoes. *Journ. Agric. Research*, xix, No. 7.
- (7) — (1923). Transmission, Variation and Control of Certain Degeneration Diseases of Irish Potatoes. *Ibid.* xxv, No. 2.
- (8) — (1925). Infection and Dissemination Experiments with Degeneration Diseases. Observation in 1923. *Ibid.* xxx, No. 6.
- (9) SMITH, KENNETH M. (1926). A Comparative Study of the Feeding Methods of Certain Hemiptera and of the Resulting Effects upon the Plant Tissue, with Special Reference to the Potato Plant. *Ann. App. Biol.* xiii, No. 1, February.
- (10) STOREY, H. H. (1925). The Transmission of Streak Disease of Maize by the Leaf-Hopper *Balclutha mbila*. Naude. *Ibid.* xii, No. 4, November.

## EXPLANATION OF PLATES VIII—X

## PLATE VIII.

Fig. 1. View of one of the experimental plots showing cages in position. Each cage is held firmly in place by an external wooden frame.

Fig. 2. Successful transmission of mosaic disease by means of the aphid *Macrosiphum (solanifolii) gr.* This plant commenced with mild mosaic, but at the time the photograph was taken late in the season, it was in a curly dwarf condition.

Fig. 2 A. Healthy control plant.

## PLATE IX.

Fig. 3. Successful transmission of mosaic by means of the aphid *Myzus persicae*.

Fig. 3 A. Healthy control plant.

## PLATE X.

Fig. 4. Successful transmission of mosaic by means of the leaf-hopper *Zygina pallidifrons*.

Fig. 4 A. Healthy control plant.

Fig. 5. Leaves from plant in Fig. 4 enlarged to show mosaic symptoms. All the plants are variety "President."

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# THE LIFE HISTORY AND BIONOMICS OF A BRITISH PHYTOPHAGOUS CHALCIDOID OF THE GENUS *HARMOLITA* (*ISOSOMA*)

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(With 12 Text-figures.)

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## I. INTRODUCTION

ALTHOUGH many economically important species of the genus *Harmolita* have been studied in different parts of the world, notably in the United States of America and in Russia, nothing is known of the biology of this genus in Great Britain. It was therefore considered desirable to investigate the life history and biology of British species of *Harmolita*.

Several species of *Harmolita* which are gall formers on the common couch grass (*Triticum repens*) proved to have a very wide prevalence in this country and consequently formed a convenient source of material for this investigation.

The life history of one of these species attacking *Triticum repens*, namely *Harmolita graminicola* (Gir) has been described with full details; one other undetermined species whose larva is also gallicolous on couch grass has been figured. Its life history approximates very closely to that of *Harmolita graminicola*.

The latter species was successfully induced to breed in captivity on *Triticum repens* but refused to breed on wheat or any other available representative of the *Triticum* genus.

It is quite possible however that closely allied species may be gall formers on cultivated wheat although I have not yet succeeded in finding the wheat plant infested with species of *Harmolita* in this country.

So far, also, none of the British species of *Harmolita* examined exhibited the interesting phenomenon of complete change of diet reported by several investigators concerning some foreign species of this genus. Thus Rimsky-Korsakov(5), whilst working on the Russian *Harmolitæ* attacking cereals found that one of his species named *Harmolita inquilinum*, oviposits in the galls of another species of the same genus (*H. rossicum*), and that its larva feeds first on the larva of the gall maker and then finishes its development phytophagically. Phillips(4) reports that the larva of *Eurytoma pater*, a genus closely allied to *Harmolita*, is a true parasite in its early stages of the larva of an *Harmolita*, and that it consumes the host larva before completing one-third of its own growth and then finishes its development on plant tissue. Nielsen observed a similar phenomenon in another Eurytomid.

These facts are considered by Gahan(1) to suggest that phytophagy in Chalcidoidea is a recent specialisation forced upon the chalcid parasite by a premature exhaustion of the natural food supply. This research was commenced in the Zoological department of Manchester University, and completed in the Zoological section of the department of Agriculture in the Leeds University. I must here express my obligation to Professor S. J. Hickson, F.R.S., of Manchester, and Professor R. S. Seton, and Mr T. H. Taylor of Leeds, for facilities to carry out this work. My thanks are also due to Miss E. M. Wright and to Miss M. Jepson for drawing some of the text-figures.

## 2. METHODS OF INVESTIGATION.

The fact that the whole of the post-embryonic developmental period of species of *Harmolita* is passed enclosed within galls is productive of certain difficulties in the investigation of individual life cycles. The opening of a gall containing a larva in one of its trophic stages is tantamount to the latter's destruction, even though the minute and delicate creature be uninjured in the process. This is due to the drying up of the tissues of the gall which become no longer acceptable as food to the larva.

Hence it is impracticable to follow any one specimen through the



various stages of its life cycle. When, however, a larva has become full fed and commenced to hibernate it may be removed from the gall as the latter is then only required for protective purposes. It was found that when hibernating larvae were removed from their galls, and enclosed under an inverted watch-glass upon a sheet of filter-paper they would develop normally if kept in the dark and left at the prevailing seasonal temperatures.

Galls collected in October, when the great majority of the larvae had commenced to hibernate and afterwards maintained in the laboratory at a fairly uniform temperature of about 60° F. produced a remarkable acceleration in their rate of development in response to the stimulus of higher temperatures. Emergence of the adults could be made to take place as early in the year as February, which was about four months before the normal time.

Rimsky-Korsakov (5), observed a somewhat similar phenomenon when studying Russian species of *Harmolita*. Whilst studying the stages subsequent to hibernation, this method of removal from the galls was found superior to that of continually taking out and replacing larvae in the galls, for however careful the operator may be, larvae are liable to be killed or so injured that a retardation or distortion of growth takes place.

In order to study the early stages of the life cycle *Harmolita graminicola* was successfully bred in captivity. The procedure adopted was as follows: "Roots" of couch grass (*Triticum repens*) bearing young shoots were transplanted into fairly large plant pots with a diameter of about a foot and a height of about a foot and a half. Ten of these pots were employed and each contained numerous young shoots of *Triticum repens*. Several precautions are necessary for success. First, if the inflorescence has burst through the sheathing leaves no attempt at oviposition will be made on the grass culm. Second, transplanting should be undertaken well before the time of emergence in order to give the plant time to get over its ill effects and to ensure that eggs have not already been deposited in its culms.

The plants were then enclosed in glass cylinders whose open tops had been covered with muslin. The cylinders have a height of about two feet and were of such a diameter as to fit easily on the tops of the plant pots. The pots were kept during the breeding experiment in an open insectary at the Manchester University Experimental Grounds at Fallowfield. The insectary has a corrugated iron roof, but the sides are only protected by a large mesh wire netting thus ensuring natural conditions of light and temperature.

Galls were collected as near to the time of emergence as possible and the adults allowed to emerge under glass cylinders similar to those enclosing the potted grass plants. After emergence the insects were left undisturbed for three days in order to ensure maturation and fertilisation (if the latter is really necessary) of the eggs. At the end of this period the cylinders enclosing the plants were removed and replaced by cylinders containing the adult insects.

Oviposition then took place. The insects were allowed to remain in contact with the plants for 24 hours; this period of time was found sufficiently long to ensure oviposition.

### 3. TIME OF EMERGENCE AND LONGEVITY OF IMAGINES.

The period of normal emergence of the adults extends from about June 24th to July 7th. The imago escapes by eating out a circular hole in the wall of the gall with its mandibles. The aperture through which it emerges is not confined to any particular spot on the wall of the gall. The first act of an imago after emergence is to remove particles of plant debris from off its body and limbs. This done the insect proceeds to rear itself up on the posterior apex of its abdomen and to work off, by means of its prothoracic legs, the old pupal envelope still encasing each antenna. At least an hour elapses before the newly emerged insect shows a desire to use its wings. The life of the imagines may be of considerable length for they have been kept alive in captivity without access to food or water for 14 days. Those which oviposited in the breeding cages did not die soon afterwards but appeared to live about as long as the males in whose company they were kept. Probably a month represents the normal duration of life of the adult *Harmolita*. They were observed when at liberty feeding on the pollen of various grasses which appears to constitute their chief source of food.

### 4. PARTHENOGENESIS.

The evidence is strongly against the occurrence of parthenogenesis in *Harmolita graminicola*, although there is still some doubt concerning the matter. The sexes occur roughly in about equal numbers during the only generation of the year. Several experiments were conducted in an endeavour to determine with certainty whether fertilisation was necessary. A number of galls were opened shortly before the time of emergence to ascertain the sex of the occupants. A number of female pupae were collected in this manner, placed in breeding cages and allowed to mature. The resulting female flies without being afforded an

opportunity of fertilisation were allowed to come into contact with *Triticum repens*. Extensive oviposition took place but no galls subsequently resulted. These results, however, may still be regarded as inconclusive, and a repetition would be desirable especially in view of the fact that the breeding of these chalcid flies is a somewhat delicate operation, and some other factor distinct from non-fertilisation may have intervened to prevent the development of the eggs.

### 5. OVIPOSITION.

The opportunities of watching the act of oviposition were many and the method of egg laying appeared to be as follows:

The fly crawls along the grass culm tapping it at frequent intervals with its antennae. The latter organs appear to be acting in an investigative capacity. When an apparently suitable spot is reached the front end of the body is raised and the hinder end lowered until the first two pairs of limbs hang free and the posterior tip of the abdomen is in contact with the surface of the grass culm, the insect being pivoted on its metathoracic legs. The two palp-like continuations of the inner plates can be seen adjusting the tip of the ovipositor sheath to the exact spot where the puncture is to be made. The ovipositor sheath with its contained stylets are then driven into the plant tissue by a backwardly directed sliding movement of the abdomen. The sheath by this movement is drawn away from its customary position along the ventral surface of the abdomen and makes an angle of from  $45^{\circ}$  to  $90^{\circ}$  with the ventral surface of the abdomen. The ovipositor sheath is now driven its full length into the plant by the approximation of the ventral surface of the abdomen to the leaf sheath.

The time during which the ovipositor remains inserted varies. The writer has seen its retention in the plant for the period of a minute but more frequently it has been withdrawn after 10 seconds. During the time of insertion the ovipositor is partially withdrawn and pressed in again several times. Only one egg is laid in each grass culm, and this was shown to be invariably the case by a series of dissections of grass culms immediately after oviposition in them.

The egg is always deposited in the centre of the stalk about 2.5 mm. below the tip of the rudimentary inflorescence. The egg consists of an elongated oval body with a fairly long pedicel at one pole and a very short rudimentary one at the other. The chorion is smooth and transparent and the developing embryo can be seen quite well within, under suitable optical conditions. The length of the body of the egg excluding

the pedicel is generally just a little short of 0.4 mm. The body of the egg passes anteriorly down the ovipositor, and its passage is thus facilitated, as part of the contents of the body appears to pass into the pedicel during the passage down the ovipositor. No part of the egg protrudes beyond the epidermis after oviposition has taken place.

#### 6. PERIOD OF INCUBATION.

After oviposition had taken place in the manner described above, endeavours were made to ascertain the length of the period of incubation. Dissection of a culm containing an egg was made daily from each of five pots used for this experiment for a period dating from oviposition to the day a larva was found. If it happened that a grass culm did not contain an egg another one was taken from the same pot until one containing an egg was discovered. On the day a larva was first found all the remaining culms in that particular pot were dissected.

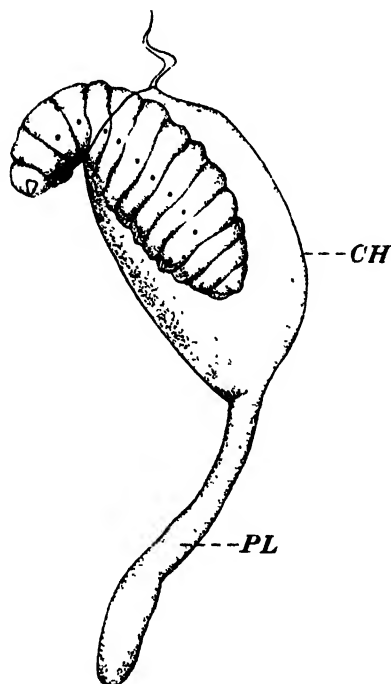


Fig. 1. The eclosion of the larva from the egg  $\times 120$  diams. Drawn from a Canada balsam mount stained with Delafield's Haematoxylin. The larva has shrunk proportionately more than the egg membrane.

CH, Chorion of egg. PL, Pedicel of egg.

The period of incubation was found to be a comparatively long one as the following table shows:

Pot number	Date of oviposition	Grass culms dissected	Eggs dissected out	Date larva first found
1	June 30th	37	27	July 24th
2	July 1st	30	23	—
3	„ 1st	42	15	—
4	„ 1st	35	27	July 23rd
5	„ 1st	44	29	„ 27th

The above table shows pretty conclusively that the period of incubation lies between three and four weeks for *Harmolita graminicola*. In Pot 1, as shown by the table, the first larva was dissected out on July 24th and from the remaining culms on the same day four eggs and five larvae were obtained. From Pots 2 and 3 all the eggs were dissected out before the period of incubation was over, consequently no larvae

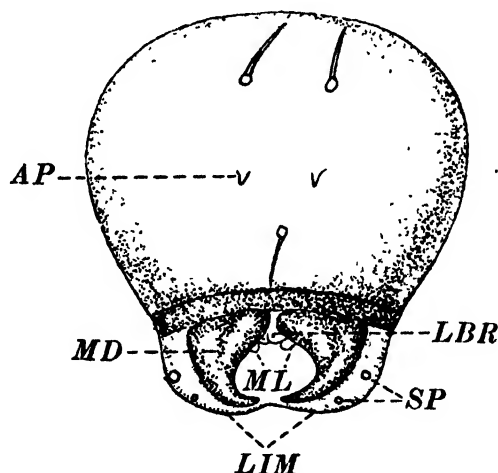


Fig. 2. The frontal view of a cephalic segment of a newly hatched larva about 0.5 mm. long, showing the complete absence of sub-apical teeth on the mandibles  $\times 300$  diams. Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

AP, Antennal papilla. LBR, Bi-lobed labrum bearing two sense spots. LIM, Bi-lobed labium. MD, A mandible. ML, Maxillary lobes. SP, Sense spots of labium.

were obtained from these pots. In Pot 4 the first larva was found on July 23rd, and from the remaining culms dissected out on the same day five eggs and four larvae were obtained.

In Pot 5 the first larva was found on July 27th, and from the remaining culms three eggs and seven larvae were obtained. The eggs examined in the fourth week after oviposition showed unmistakable

signs of the nearness of hatching and the rapidly developing larva could be seen within. The body of the egg increases in size as the time of hatching approaches and a tension is set up in the egg membranes. The larva escapes from the egg at the pole opposite to that at which the long pedicel is situated. The larva does not possess any specialised

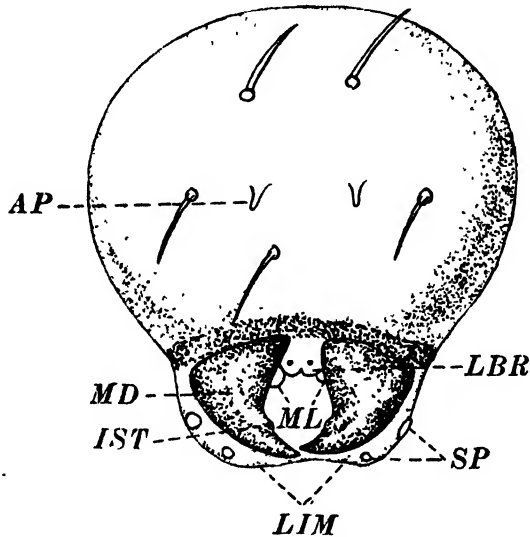


Fig. 3. The frontal view of a cephalic segment of a larva of the second instar, measuring about 1.3 mm. long, showing the incipient sub-apical tooth of the mandibles  $\times 200$  diam.

*IST*, Incipient sub-apical tooth of a mandible. Other lettering as in Fig. 2.

Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

hatching apparatus and appears to rupture the already tense chorion by means of its mandibles assisted no doubt by movements of the body.

Fig. 2 shows a larva in the act of escaping from the transparent egg membranes and the part of the larva still within the egg could be clearly seen.

## 7. THE LARVA OF THE FIRST INSTAR.

The newly hatched larva varies in length from 0.4 to 0.5 mm. and is usually found adhering to the empty egg-chorion. The segmentation is clearly defined, and consists of a head and 13 body segments. The cephalic segment is smaller in proportion to the segments immediately behind it than in larvae of later instars, and the antennae papillae seem to be more conspicuous.

The hinder part of the body tapers more gradually and is more pointed than in older larvae. Unlike many parasitic chalcid larvae the alimentary canal is continuous from mouth to anus from the time of hatching as expulsion of liquid excrement from the anus was observed almost immediately after hatching. It is easy to ascertain whether a larva has begun to feed, because if food has been taken the mesenteron

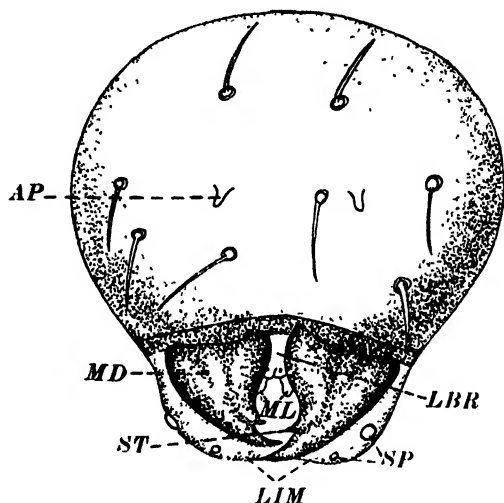


Fig. 4. A frontal view of a cephalic segment of a larva of third instar about 2.1 mm. long, showing the development which has taken place in the sub-apical tooth of the mandible  $\times 150$  diams. Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

ST, Sub-apical tooth of the mandible. Other lettering as in Fig. 2.

will exhibit a distinctly green colour which can be observed very clearly through the almost transparent body. Little lobulated masses, slightly more translucent than the general content of the body can be discerned here and there beneath the transparent integument. These bodies form the basis of the large mass of fat body which subsequently develops and finally renders the larvae of the later stages completely opaque.

The respiratory system is the same fundamentally as in larvae of later stages; it consists of a pair of longitudinal tracheal trunks united transversely, both anteriorly and posteriorly by commissures.

The longitudinal trunks give off at segmental intervals lateral branches to the spiracles. The spiracles can be recognised as nine in number on each side, a pair being situated on each segment from the second to the tenth inclusive. The respiratory system is peripneustic from birth with closed spiracles on the wing-bearing segments. The air

tubes show up clearly in freshly dissected larvae of this instar owing to the relative absence of fat body. By suitable staining various internal structures such as the salivary glands and central nerve cord can be observed. Faint indications of the cutaneous muscular system can also be seen. The feature distinguishing the first instar larva is found in the mouthparts.

The mandibles (Fig. 2, *MD*) do not each possess a sub-apical tooth which is present incipiently at least in all later instars (Fig. 3, *IST*, and Figs. 4 and 5, *ST*). There is a prominent slightly bi-lobed labrum

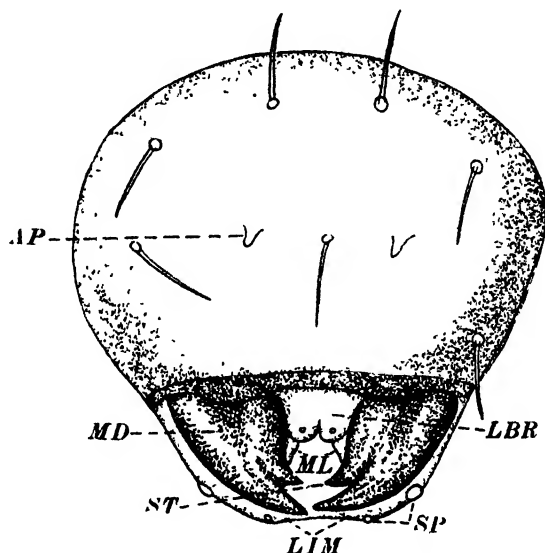


Fig. 5. A frontal view of a cephalic segment of a mature larva showing the fully developed sub-apical tooth of the mandible · 130 diams. Drawn from a potashed mount stained with 2 per cent. acid fuchsin

*ST*, Sub-apical tooth of the mandible. Other lettering as in Fig. 2.

(Fig. 2, *LBR*) bearing a small sense spot on each lobe. A pair of maxillae are also represented by two lobes (Fig. 3, *ML*). The labium (Fig. 2, *LIM*) is very large and indented slightly in the median line to give it also a somewhat bi-lobed appearance. Each of these lobes bears two sense spots (Fig. 2, *SP*) one of which is relatively very large and presents a hemispherical face.

A larva after it has been feeding for a day or two may be found in a little cavity eaten out by itself just beneath the germ of the future inflorescence which is destined never to shoot the enveloping sheath and ripen into seed. Whilst growing to about 1 mm. in length a larva does



not produce any external indication of its presence within the stem. Subsequently an elongated swelling begins to form above the uppermost node of the stalk and when the larva is 2 mm. long it is quite apparent.

#### 8. INSTARS LATER THAN THE FIRST.

The first moult takes place when the larva is about one week old and measures about 1.3 to 1.5 mm. long. Apart from size and a slightly greater translucence of the body content the larva of the second instar

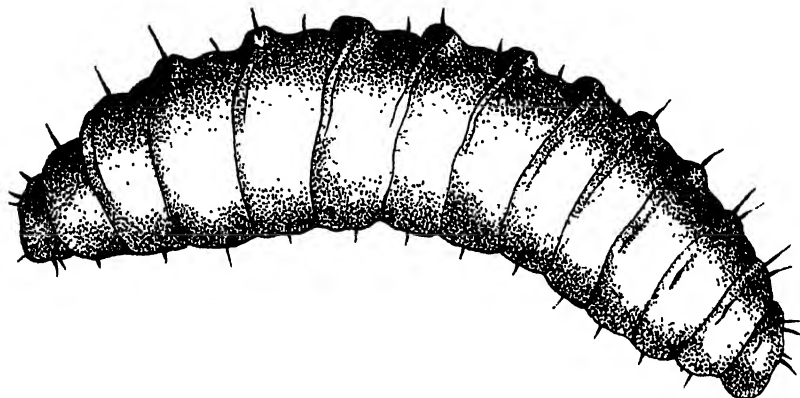


Fig. 6. A side view of a mature larva of *Harmolita graminicola* (Gir)  $\times 50$  diams.

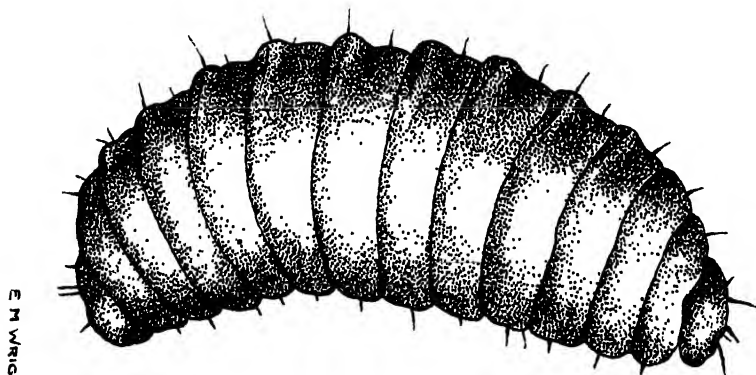


Fig. 7. A side view of a mature larva of an undetermined species of *Harmolita*  $\times 50$  diams.

differs from that of the first only in that the mandibles of the former exhibit indications of sub-apical teeth (Fig. 3, *IST*). The second stadium lasts about a fortnight when another ecdysis is effected, the length of the larva being then about 2.0 to 2.2 mm. In this, the third instar of

the larva, the sub-apical teeth of the mandibles (Fig. 4, *ST*) are much better developed. The third stadium also lasts about a fortnight and when the larva measures about 3 mm. long the cuticle is cast for the third time.

This is the last moult prior to the one at pupation. In the last instar the sub-apical teeth of the mandibles are fully developed (Fig. 5, *ST*). The body contents have become quite opaque owing to the increase in size, number, and density. In the month of October the great majority of the larvae reach the fully fed condition and they then enter into a very quiescent state. In this state of hibernation the fully fed larva passes the winter under the protection of the gall.

#### 9. THE PRO-NYMPH OR SEMI-PUPAL STAGE.

Pupation begins to take place about the end of April but at least 90 per cent. pupate in the middle of May. As the time of pupation draws near the old larval skin begins to lift in preparation for the moult. About 48 hours before pupation takes place, the larval body assumes a permanent flexure at the region of the third and fourth body segment, and the sternal region of the fourth segment becomes almost obliterated. About this time a series of rhythmic risings and fallings of the larval cuticle can be detected. These are brought about by the circulation of the moulting fluid between the larval cuticle and the new pupal cuticle beneath. They are specially discernible in the lateral abdominal region in the neighbourhood of the spiracles where a ridge-like puckering is formed by repeated risings and fallings of the cuticle.

The moulting fluid after permeating between and aiding in the separation of the old larval cuticle, from the underlying coat, is gradually worked by a series of wavelike motions to the dorsal region of the thorax. The cephalic and thoracic segments become swollen and paler in colour owing to the pressure caused by the underlying accumulation of fluid. This is the true semi-pupal stage corresponding to that described in some other Hymenoptera (3), and lasts from 12 to 24 hours in this case. The moulting fluid first causes the swelling or "blister" on the whole of the dorsal thoracic region, but later the fluid is forced forward and heaped up over the dorsal prothoracic region and the tension in the cuticle at this spot becomes very great. Then by an increase in the flexure of the body at the region of the third and fourth segments already referred to and by vigorous wriggling in the abdominal region the breaking point in the swollen prothoracic region is reached. The rupture of the old larval integument which begins in the median line of the

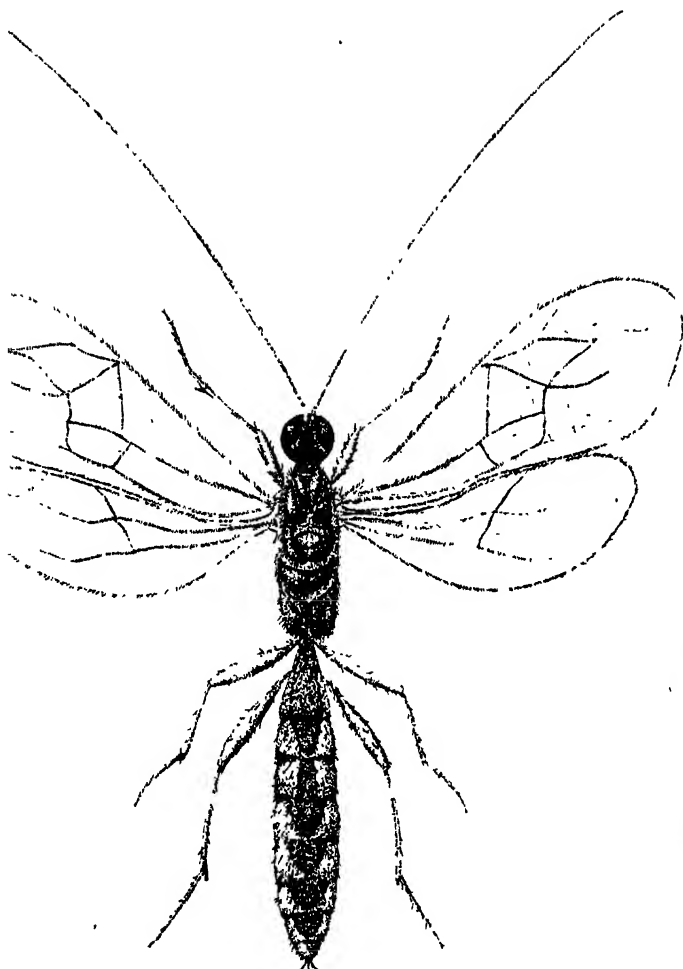


Fig. 8.



Fig. 9.

Fig. 8. Female adult of *Bracon erythrostictus* (Lyle) parasitic on *Harmolita* in the larval stage  $\times 50$  diams.

Fig. 9. A parasitised larva of *Harmolita graminicola* *in situ* in the gall  $\times 10$  diams.

dorsal prothoracic region rapidly extends in a longitudinal direction over the whole length of the dorsal surface of the thorax and is followed by a liberation of the moulting fluid and the rapid emergence of the pupal head which lay in the prothoracic region of the larva.

The old larval integument with the mandibles attached is gradually worked towards the posterior region of the body by repeated movements of the abdomen and by occasional flexion of the entire body about the fourth segment. When isolated from the gall the act of slipping out of the larval cuticle takes from 40 minutes to an hour, and the process may take a considerably shorter time inside the gall. Finally the old larval skin is forced back to the posterior extremity of the abdomen where it remains attached almost throughout the pupal stage as a dried appendage attached to the posterior extremity of the abdomen.

#### 10. THE PUPAL STAGE.

Immediately after pupation has taken place the pupa presents a very pale yellow colour and no black markings are discernible. For some little time after the pupal moult the pupa is sticky to the touch owing to the moulting fluid and there is a palpitation on the vertex combined with frequent movements of the abdomen. The new pupal integument

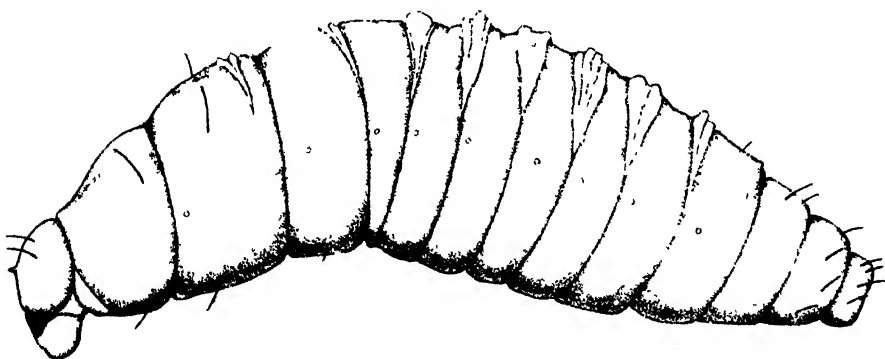


Fig. 10. The larva of *Harmolita* in the stage immediately preceding the semi-pupal stage  
× 300 diams.

is very soft and flexible, but the general conformation of the chief sclerites of the adult can be seen. The pedicel or "waist" is marked only by a very slight constriction and the segmentation of the abdomen is very faint.

There is a well-defined ridge on the head just ventral to the insertion of the antennae, and beneath this ridge is a swelling which represents the

rudiments of the labrum. Below the latter comes the rudiments of the mandibles followed by the maxillae and labium with their palps. The legs and antennae are glued to the ventral surface but their segmentation is very indistinct, only the limits of the coxae on the legs and the scapes on the antennae being demarcated.

The male pupae are easily separated from the female by reason of their markedly longer antennae. The mesothoracic wing cases are glued against the body and hide the metathoracic wing cases. The compound eyes are present just after pupation but no ocelli are visible at this stage. A pronounced lateral ridge is present on each side of the abdomen which extends from the propodeum to the posterior extremity of the abdomen.

At about four days old the pedicel becomes better defined owing to an increase in the constriction; the cuticle becomes harder and indications have appeared of the two dorsal ocelli. The darkening of the pupa begins on the eighth day of the pupal stage. A dark brown streak appears in the median line near the mouth region. Soon afterwards black pigment is deposited on the compound eyes, on the mandibles, on the intersegmental regions of the abdomen, and on the three ocelli which are now visible. This blackening process continues until by the sixteenth day of pupation the pupa is black in colour. Meanwhile the pedicel becomes still more constricted and the segmentation of the legs and antennae more distinct. Chitination of the imaginal integument proceeds and the outer pupal coat becomes dry and loosely attached to the hardening imaginal coat beneath.

The length of the pupal period is about 40 days, but this can be reduced to 28 days by allowing pupation to take place in an artificially heated laboratory.

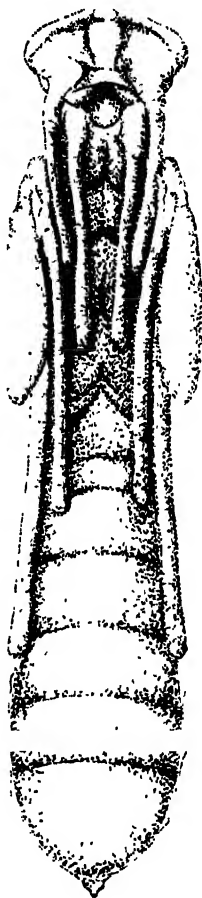


Fig. 11. The pupa about the middle of the pupal period  $\times 50$  diams.

## 11. NATURAL ENEMIES.

Although many galls produced by *Harmolita graminicola* have been examined, only one species of insect parasite was discovered and its degree of infestation would not exceed 1 per cent. This parasite attacks the larval stage and it was determined as *Bracon erythrostictus* (Lyle) (Fig. 8).

The larva of *Harmolita* is parasitised at an early stage within the dried skin of the larva which assumes a brownish colour (Fig. 9). *Bracon*

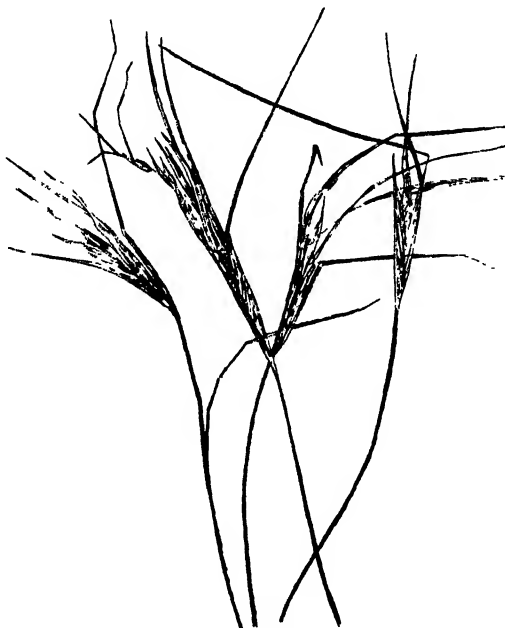


Fig. 12. View of the galls made by larvae of *Harmolita* on *Triticum repens* about natural size.

*erythrostictus* emerges about a fortnight to three weeks before *Harmolita* appears. The former parasitises both species of *Harmolita* dealt with in this paper. The parasites of *Harmolita* are recognised as of great economic value in the United States of America as they help very largely to keep jointworm in check in normal years.

The only other natural enemy of any importance were certain birds. Large numbers of larvae are pecked out of galls by birds, particularly by tits; especially if the winter is severe.

## 12. SUMMARY.

This paper is a contribution to our very scanty knowledge of the British *Harmolita*, a phytophagous genus of the superfamily Chalcidoidea (Hymenoptera). Many species of this genus are pests on cereals and cultivated grasses; one species, *Harmolita tritici*, is the notorious joint-worm of America and Russia, whilst species attacking many other cereals and grasses have been recorded in Europe and America. An account is here given for the first time of the life history and biology of a British species of *Harmolita*, namely *Harmolita graminicola*, which is a gall former on couch grass (*Triticum repens*).

The larva of another undetermined species of *Harmolita*, also gall-colous on *Triticum repens* is figured and briefly described.

The adult of *Harmolita graminicola* begins to emerge in the last week of June and continues to appear during the first week of July. When the inflorescence has appeared through the sheathing leaves the female fly will not lay eggs on them. One egg is deposited on each culm just beneath the rudimentary inflorescence, the ovipositor of the fly piercing the sheathing leaves in order to reach the desired position. The *Harmolitae* of both species were bred in captivity and the length of the period of incubation of the egg was found to be between three and four weeks. Experiments were conducted to ascertain whether parthenogenesis occurred in this species. The results were negative although perhaps not numerous enough to be conclusive.

The larva possesses four instars and moults three times whilst feeding, another moult occurring at pupation. The larval instars can be differentiated most exactly by the state of development of the sub-apical tooth on the mandible. The sub-apical tooth is absent from the mandibles of the first instar; only incipiently developed in the second instar; better developed in the third and fully formed in the fourth instar. The larva becomes full fed in October and hibernation takes place in the larval stage. Pupation begins to occur near the end of April but the great majority pupate in the middle of May.

There is a short semi-pupal stage of from 12 to 24 hours duration immediately before the pupal moult takes place. The pupal stage lasts about 40 days and there is only one generation each year. The larva of *Bracon erythrostictus* (Lyle) was found to be parasitic on *Harmolita* in the latter's larval stages; no other insect parasites were found. Both *Harmolita graminicola* (Gir) and the undetermined species refused to breed on wheat or any other plants of the *Triticum* genus although many attempts were made to induce them to do so.

## REFERENCES.

- (1) GAHAN, A. B. (1922). A list of Phytophagous Chalcidoidea with descriptions of two new species. *Proc. Ent. Soc. Wash.* February, XXIV, 34-38.
- (2) IMMS, A. D. (1916). Observations on the insect parasites of some Coccidae. I. On *Aphelinus mytilaspides*, Le Baron, a chalcid parasite of the mussel scale (*Lepidosaphes ulmi*). *Q.J.M.S.* LXL, 217-274. Five figures and Plates XIX and XX.
- (3) — (1916). *Ibid.* II. On the Chalcid parasites of *Lecanium caprea*. *Ibid.* LXIII, 1918, 293-374. Thirty-four figures.
- (4) PHILLIPS, W. J. (1920). Studies of the life history and habits of the jointworm flies of the genus *Harmolita* (*Isosoma*) with recommendations for control. *Bull.* 808. *U.S. Dept. of Agric. Wash.*
- (5) RIMSKY-KORSAKOV, M. N. (1914). Chalcids of the genus *Isosoma*, injurious to grain crops in Russia. *Memoirs of the Bureau of Entomology of the Scientific Committee of the Central Board of Land Administration and Agriculture, St Petersburg*, x, No. 11, 84 pp. Fifty figures, three plates. (*R.A.E.* Series A, vol. II, 1914, p. 470.)

(Received June 12th, 1926.)



## REVIEWS

*The Theory of the Gene.* By THOMAS HUNT MORGAN. Yale University Press, 1926. Pp. xvi + 343, Figs. 156. 18s. net.

This volume is an outline of the theory of genetics from the point of view of the American worker on *Drosophila*. Its wide scope is shown by the chapter headings which are as follows: I. The fundamental principles of genetics. II. Particulate theories of heredity. III. The mechanism of heredity. IV. Chromosomes and genes. V. The origin of mutant characters. VI. Are mutant recessive genes produced by losses of genes? VII. The location of genes in related species. VIII. The tetraploids, or fourfold type. IX. Triploids. X. Haploids. XI. Polyploid series. XII. Heteroploids. XIII. Species crossing and changes in chromosome number. XIV. Sex and genes. XV. Other methods of sex-determination involving the sex-chromosomes. XVI. Intersexes. XVII. Sex reversals. XVIII. Stability of the gene. XIX. General conclusions. Bibliography. Index.

The chromosome interpretation of the mechanism of heredity is accepted throughout and fresh data in support of it are brought forward. Chapters I-IV are essentially introductory. Chapter V is all too short for the critical importance of its subject and leaves one with a curiously unsatisfied feeling. It is a pity that the opportunity was not taken to give an exact and unequivocal statement of what the term "mutation" connotes. So much controversy has arisen from the different usages of this word, and there are such varied and discrepant definitions of it in recent genetical treatises, that a precise statement by Prof. Morgan, which, one might expect would be adhered to by American workers, would do much to clear up the confusion.

Chapter VI in which the "Presence and Absence Theory" is discussed adversely is of considerable interest, but certain data such as those relating to the albino guinea-pig, the recessive black-rabbit, the white factor in the jungle-fowl and the bar-eye in *Drosophila* are capable of interpretations other than those adopted.

Chapters VII-XIII dealing with various aberrant and multiple chromosome arrangements are a masterly summary of the very widely scattered data in this field. One expected however to find some reference to the recent criticisms of Jeffrey and Hicks, who are not mentioned, and of Lotsey to whom only two references, neither later than 1916, are given.

Chapters XIV-XVII, in which problems of sex are considered, are perhaps the most interesting portion of the volume although not so coherent in treatment as the previous section. The discussion of intersexes and sex-reversals in both animals and plants is particularly useful. It may perhaps be still premature, but one would have liked to see included reference to the striking cases of sex-reversals in fungi.

Chapter XVIII on the stability of the gene is exasperatingly brief and gives little idea of the present position or perspective of the problem. One would have liked a much fuller discussion of the "inheritance of acquired characters," more particularly from the plant aspect; to find reference to Kammerer, Tornier, Guyer, etc.; to see some discussion of this question in relation to bacteria, fungi and protozoa, where it is of primary importance and where evidence is rapidly accumulating. The causes or stimuli of changes of gene are still as obscure as ever in the higher forms, but, in micro-organisms, they seem to be more within our reach. The data from all fields of study, however, seem to point more and more clearly to the view that "mutation" is no fortuitous happening but is definitely causal in nature, a specific and orderly process.

The present book constitutes the nineteenth volume published on the Silliman Lectures Foundation of Yale University. The only other book in this series of general

biological interest is the eighth volume which is Bateson's *Problems of Genetics*, published in 1913. If one compares these volumes one obtains a vivid impression of the changes in sheer amount of data (almost the whole of the *Drosophila* work) in values and in perspective that thirteen years of intense genetical research have produced. Also, incidentally, one cannot help being struck by the difference between the big philosophic outlook and synthetic grasp of Bateson's volume, and the widely ranging but far less coherent and more intensive outlook of Morgan's volume.

Viewing the present book in the light of the past thirteen years of research, which is just one-half the age of modern genetics, one cannot help feeling that fresh points of view are needed in this field of work. For twenty-six years genetic research has been almost entirely a matter of cytological detail and of juggling with Mendelian factors in experimental breeding; the physiological bases of the genotype and the environmental conditioning of the phenotype have been neglected. In very few genetics' laboratories are there first-class physiologists at work and yet these could do much to widen the present canalised approaches to genetical problems and to drive new avenues.

The volume contains a very useful bibliography which would have been still more useful if it contained references to more of the authors mentioned by name in the text. For example—and this is only one of many in the book—in discussing *Tetranychus bimaculatus* the references given are "(Perkins, H. A. Morgan, Bank, Ewing, Parker)," not one of whom is mentioned in the bibliography. The volume also apparently contains errors on pages 12, 113 and 293 and in Figs. 11, 12 and 141, but otherwise it is beautifully produced, both printing and binding, and the illustrations are admirable.

*The Theory of the Gene* is an up-to-date presentation of the views of one of the most eminent of living geneticists and taken in conjunction with his *Mechanism of Mendelian Heredity* (1923), and his recent detailed monograph on the *Genetics of Drosophila* (1925), it gives one a very good idea of the present position of the monumental researches that are steadily taking shape in the Columbia laboratories and of the lines of thought that are crystallising out in them. No research worker in applied biology can afford to neglect these more theoretical aspects of genetics, for they underlie all practical problems of disease, of crop growth, animal husbandry, economic breeding and the like.

WILLIAM B. BRIERLEY

*Life of Plants.* By Sir FREDERICK KEEBLE. Clarendon Press, Oxford, 1926. Pp. 256, Figs. 51. 5s. net.

Since *Chapters in Modern Botany* by Geddes, the only botanical pocket volumes that one remembers as worth while are Praeger's *Open Air Studies in Botany*, the volumes by Scott and Farmer in the Home University Library, the Cambridge Manuals by Seward and Bower and Dixon's lectures. The present volume bears comparison with these. It is packed full of meat and yet so easily is it written and so apt are the allusions and illustrations that the reader is almost unaware of the strength of his diet. *Life of Plants* is an Essay in Botanik rather than a botanical text-book and presents essentially a personal point of view. Many a botanist might quarrel with the author's perspective and balance of values, his avoidance of disease and death which are a too often neglected aspect of plant life, the little attention given to the dominance of gametophyte or sporophyte phases in different plant groups, the confident acceptance of hormones to explain unification of behaviour, the neglect of the more "social" aspects of plant life and so forth. That one finds so many points on which to differ is a tribute to the unusual and stimulating quality of the book. The volume is finely printed and bound and the illustrations are refreshingly original.

WILLIAM B. BRIERLEY

*Contributions from the Harvard Institute for Tropical Biology and Medicine, III, 1926.* Cambridge (Mass.): Harvard University Press; London: Humphrey Milford, Oxford University Press. 4to; 110 pp. and 4 Plates. Price 11s. net. I. Report on Sugar-cane Borers at Soledad, Cuba, by G. SALT. II. Dry-Season Studies of Cane Homoptera at Soledad, Cuba, by J. G. MYERS.

The first of the above articles deals with observations on sugar-cane borers made during a period of about ten weeks at Soledad, Cuba. As might be expected, the brevity of the time devoted to so comprehensive a subject did not allow for any very detailed studies to be made. Although there are in Cuba a number of scientific workers engaged upon various problems, very few are entomologists, and the need for information respecting borer attacks is urged as the excuse for what would otherwise have been regarded as a too hastily compiled publication. It appears that the 1924-25 sugar-cane crop at Central Soledad was infested by moth borer (*Diatraea saccharalis*) to the extent of 18.5 per cent. of all canes arriving at the mill. It is claimed that actual counts made at the mill are more reliable than field counts involving the same amount of time and work. There is, however, a wide range in the percentage of infestation of this insect in the various colonias or plantations: in one colonia only 5.7 per cent. infestation is recorded while the highest infestation amounted to 33.36 per cent. The distribution of the attacks appears to be influenced by topography, hills being infested to a lesser degree than valleys, and the high-land colonias show a lower percentage of attack than low-lying colonias. As an explanation it is suggested that since the *Diatraea* seems to have a preference among wild plants for aquatic grasses, the physiological state of sugar-cane in low lands and valleys more nearly simulates that of such grasses and suffers heavier depredation in consequence. In view of the fact that natural control by means of parasites, although definitely beneficial, does not sufficiently dominate the situation, various control practices are recommended. Of the several measures advised it is interesting to note that the burning off of infested cane fields before cutting, and also of the trash after cutting, is recommended to be discontinued. It has been shown in Louisiana that trash burning is very detrimental in destroying great numbers of a beneficial egg-parasite without any commensurate advantage resulting, and the same is assumed to be true in Cuba. A list of parasites is given at the end of the article and the most important is the Tachinid *Lixophaga diatraeae* which, it may be added, has been purposely introduced from Cuba into other sugar-growing countries. Among other cane-borers, the weevil borer (*Metamasius sericeus*) appears to be more serious than was previously supposed, while on the other hand injuries occasioned by *Xyleborus* sp. and by Termites are considered to be of little importance.

The second article is likewise the result of a brief sojourn at Soledad and is written by Mr J. G. Myers. His object was to study those members of the suborder Homoptera involved, or possibly involved, as carriers of mosaic disease of cane and allied grasses. The article is intended only as a preliminary study of the situation as it appears in the driest season of the year. Our present knowledge of cane mosaic transmission by insects is summarised, and it may be said that the corn leafhopper can transmit mosaic from corn to corn but does not occur in cane. *Aphis maidis* can carry the disease from other grasses to cane, and the aphid *Carolinaia cyperi* transmits the disease from sedge to cane. None of these occur normally on cane but the two last-mentioned species have been shown to migrate to cane when their weed host-plants are eliminated. The question of insect vectors has not received much attention at Soledad where it appears that *Aphis maidis* is very rare. Whether there is any other factor than diseased seed-pieces to explain most of the present distribution of mosaic at Soledad is to the author an open question. He discusses the evidence for the incrimination of other cane Homoptera and finds that there are only nine species that, under dry-season conditions, can properly be regarded as true cane inhabiting forms. There appears to be no evidence that any of these function as vectors of mosaic disease.

A. D. IMMS

*Plant Nutrition and Crop Production.* By E. J. RUSSELL. Cambridge University Press, 1926. Pp. ix + 115 with 21 Plates and 37 Text-figures. Price 12s. 6d. net.

The present volume embodies the Hitchcock lectures delivered in the University of California by Sir John Russell in 1924.

The subject matter is divided into five chapters entitled respectively, The study of plant nutrients; Positive science and exact demonstration; Decay and the living plant; The soil micro-organisms; The soil and the living plant. In the first of these the author traces the history of the study of plant nutrition from the early speculations of Thales (600 B.C.) and the equivocal experiments of Van Helmont (1620) to modern times. It was not till the dawn of the nineteenth century that the modern era of exact knowledge may be said to have begun with the publication of deSaussure's *Recherches chimiques sur la Végétation*. In 1834 Boussingault initiated exact field experiments, quickly followed by Liebig's valuable generalisations, in which the capacity of plants to utilise simple organic compounds was for the first time adequately emphasised. It was Liebig's work that inspired Lawes' experiments with artificial manures, which demonstrated the importance of phosphates and nitrogenous compounds and led to the commercial production of superphosphate.

The development of research on manurial treatment, the problems of increased yield in crop production, are here presented in the clear and logical manner of which the author is a past master. By the careful selection of detail the reader is led to the realisation of the full complexity of the problem when we attempt to grow two blades where one grew before. Far from being simple the process involves the modification of a whole chain of conditions and phenomena, in which success is dependent on the strengthening of each individual link. We are concerned not merely with the direct reaction of the species to a particular treatment, but with its effect on the associated organisms both macroscopic and microscopic, its effect on the physical and chemical constitution of the soil and on the manner in which the soil itself and the crop it bears reacts to the climatic environment. Not the least important of the variables with which we interfere is the organisation of the individual plant, the reaction of whose internal environment is modified by the changed external conditions.

The striking differences which organisms of the same genotypic constitution may exhibit in their phenotypic expression, as witness the development or non-development of supernumerary legs in the fruit fly according to the temperature of their environment, opens up a wide vista of possibilities over and above the selective action which manurial treatments and soil-amendments will inevitably exert upon the numerous genotypic strains which comprise a cultivated race, and which may only be capable of selection by physiological means.

The author recalls the old controversy regarding the relative merits of artificial fertilisers and farmyard manure, both of which are now recognised to have their appropriate place in agricultural economy. Though we have travelled far in the short period since Lawes and Gilbert conducted their first experiments, yet how great a distance has yet to be covered is shown by the wastefulness of our existing methods, strikingly illustrated in the balance sheet of calories for the Broadbalk field where, to produce food for two men, energy sufficient to feed twelve is dissipated! Further, the delicacy of the balance which has to be maintained is indicated by the comparative constancy of the carbon-nitrogen ratio under equivalent climatic conditions. Fertilisers or partial sterilisation are means of restoring the appropriate balance between chemical constituents on the one hand and micro-organisms on the other; indeed it may be questioned whether if such treatment permanently upset the balance, the consequences might not be as drastic as those which have followed the artificial disturbances by man of the balance of nature in Australia and New Zealand.

Sir John whilst rightly stressing the complexity of the problems involved indicates the lines along which further progress may be looked for, and in particular the need for investigations on the lines of pure science unhampered by the trammels that

enmesh the empiricist and technologist. In no direction may greater advance be looked for than in our knowledge of the micro-organic population of the soil, and the author envisages the time when the soil fauna and flora of cultivated lands shall be tamed in the service of man.

It would demand a volume many times the size of this work to epitomise the achievements in agricultural research, but the reader of these pages will obtain a clear idea of the trend of modern investigation and the value of pure science to the practical man. If proof of this latter were needed, no better example could perhaps be cited than the electrically charged ploughshare which appreciably reduces the frictional resistance of the soil, for this was a direct outcome of the study of soil colloids than which no subject, superficially, would appear more academic or more widely separated from the immediate interests of the farmer.

E. J. SALISBURY

*Citrus Diseases and their Control.* By H. C. FAWCETT and H. A. LEE. McGraw-Hill Book Co., 1926. Pp. 582. Figs. 205 (15 coloured). 25s. net.

Plant pathology is arriving at the stage when the amount of data available is so great that special treatises on diseases of particular host plants, or on particular aspects of disease, are not only desirable but necessary. We have had such early volumes as that of Delacroix on *Les Maladies et les ennemis des Cafeiers*, or of Watt on *The Pests and Blights of the Tea Plant*, and more recently Hiley's *Fungal Diseases of the Common Larch*, Petch's *Diseases of the Tea Bush and Diseases and Pests of the Rubber Tree*, but the present work by Fawcett and Lee on *Citrus Diseases and their Control* sets a new standard in such publications. The book itself runs to nearly 600 closely packed pages, and even so, does not include diseases due to insect pests.

The volume is divided into four parts. The first seven chapters are devoted to general considerations such as the history of citrus-disease investigations and the structure and physiology of citrus; types of citrus diseases and the inevitable outline classification of fungi; the geographical distribution of citrus diseases; conditions affecting severity and distribution of citrus diseases; general principles of prevention and treatment of citrus diseases; fungicides, disinfectants, paints and waxes; cultural operations in relation to citrus diseases.

This first portion is very unequal in treatment as though the authors had not clearly in their minds the audience they were writing for. What does stand out, however, in this very interesting discussion, is the empirical nature of much of this general knowledge, and the little we know, more particularly, of the geographical distribution of disease and of its climatic and cultural relationships. It is, of course, extremely difficult and often impossible to collate such generalised pictures from scattered publications, partly because of the different degrees of accuracy of work in different countries, and partly because in some countries the requisite workers are absent, or where they are present, the work is often done or described from such different points of view that there are few comparable features. The collaboration in the present volume of two authors of unusually wide experience has enabled them to surmount many of these difficulties. Nothing can be more valuable in this respect than the practice increasingly adopted by American specialists of visiting the different countries in which some particular crop or disease occurs, and so enabling a comparative survey of the whole situation to be made by one pair of eyes. Such a system could be most usefully grafted on to our own practice in the overseas dominions and colonies. The need is met to a certain extent in our Imperial Bureau of Mycology, which functions most efficiently as a central information bureau, exchange and clearing-house, but a vast amount of good would result, if there was more free movement and exchange between mycologists themselves in the colonies and overseas dominions. Such a visiting, loan or exchange system, would of course be very difficult to administer, but within the British Empire the opportunities are unequalled and the advantages accruing would far more than compensate for the effort.

To return to our volume, the three remaining portions are a straightforward account of the diseases of the citrus tree; part II, dealing with root and trunk diseases; part III, with diseases of branches, twigs and leaves, and part IV, with fruit diseases. It is interesting to note how many of the diseases of roots and trunk and of fruit are caused by soil-dwelling fungi. This is an aspect of plant pathology that has been vaguely realised for some time, but only recently has it assumed a prominent place. There is very little doubt that the soil is a great reservoir of disease-causing fungi, which, in numerous cases can live for long periods if not indefinitely in a saprophytic state, becoming parasites when the opportunity offers. Some years' experience of such work leads one to suspect that there are very few parasitic fungi indeed, which, able to live saprophytically, cannot sooner or later be isolated from soil. The number of different kinds of parasitic fungi present in soil is legion, and it is only a question of applying suitable methods in order to isolate them. Not only in citrus diseases, but in diseases of all crops, far more attention must in future be paid to soil microbiology.

Parts II, III and IV of the book are a most adequate summary of our knowledge, and the authors have succeeded in occupying the position defined in their preface that they "have attempted to present in this book a discussion of the present information on citrus diseases occurring in all parts of the world where citrus fruits are grown." In the consideration of all diseases spraying and cultural treatments are recommended where these are known and feasible, but the emphasis throughout is laid on the value of growing resistant or immune varieties as the only permanent and really satisfactory method of disease control.

One of the best chapters in the volume is the last on "General Problems of Deterioration and Decay of Citrus Fruit," and this points the way to developments of plant pathology to which, in this country, far more attention should be given. We are too apt to think that the work of the plant pathologist stops when the field crop is gathered, whereas it only really stops when the produce is consumed. Storage, transport, and markets' pathology are hardly known here and present a large and promising field of study.

Considering the size and nature of the book the consistently high level of writing is unusual. One cannot help liking the early-Victorian savour of the term "Internal Decline of Lemons" or appreciating the description of sour rot as "A putrid, sour, dirty, leaking, maggot-filled mass of mud." There is however loose wording on pages 118, 302, 362 and 422, and misprints have been noticed on pages 37, 38, 98, 113, 198, 276, 311, 354, 373, 506, 543 and 580.

The book is illustrated by 190 text-figures, many of the photographic reproductions being curiously flat, and occasionally (*e.g.* fig. 82 D), frankly bad. In Figure 153, the "A" and "B" referred to in the legend are not marked in the print. There are also 15 plates which contain some of the best colour photographs of plant diseases yet published. The bibliography runs to 18 pages and is an extremely useful and accurate compilation; the index is full and convenient to use. The actual format of the book has the technical excellence of all the volumes in this fine series.

The preparation of the volume, in which so much of the matter is the outcome of the authors' personal researches, is a very fine achievement, and sets a standard that will be difficult to equal. The book will immediately take rank as the authoritative and indispensable work of reference on the subject.

WILLIAM B. BRIERLEY

## OBITUARY

## PROF. F. W. GAMBLE, F.R.S.

Prof. F. W. GAMBLE, D.Sc., F.R.S., died at Alvechurch, Worcestershire, on September 14th last at the age of 57. Born at Manchester in 1869 he was the son of the late Mr William Gamble of Arnside, Westmoreland. His early education was at the Manchester Grammar School and from there he passed on to the University of that city. It was here he came under the influence of the teaching of the late Prof. Milnes Marshall, and Gamble proved one of his most successful pupils. His university studies culminated in his graduating with first class honours in zoology in 1891, and his appointment to a Berkeley Fellowship in the following year. After a period of study at Leipzig University he returned to Manchester in order to take up the post of lecturer in zoology. Gamble was a sound and successful teacher in his *alma mater*, and this had much to do with his subsequent appointment (in 1909) to the chair of zoology at Birmingham University, a post which he held up to the time of his death. In his early years as a University teacher he accomplished useful research work, and his first important memoir was on British Marine Turbellaria, which appeared in 1893. This work was followed by several researches of outstanding merit carried out in conjunction with colleagues. Mention needs to be made of the fine memoir on *Arenicola*, prepared in conjunction with Prof. J. H. Ashworth, and his experimental studies on the colour changes of *Hippolyte* and on the bionomics of *Convoluta*, both works being carried out in collaboration with Sir F. W. Keeble. After his appointment to Birmingham heavy teaching duties fell upon Prof. Gamble, and for a time he also acted as director of a small school of agricultural helminthology which had been established, in conjunction with his department, by the Ministry of Agriculture. For a short period he served as a member of the Research Council of that same Ministry. Prof. Gamble was also a member of the Council of the Association of Economic Biologists at the time of his death, but ill-health was responsible for his inability to attend its meetings. To the general student he was perhaps best known as the editor of several successive editions of Marshall and Hurst's well-known *Practical Zoology*. In 1907 he was elected an F.R.S., and in 1924 he presided over the Zoology Section of the British Association at Toronto. Prof. Gamble was married in 1904 and his widow survives him: he leaves no children.

A. D. I.

THE GERMINATION OF *POA* SPP.

By ALEXANDER NELSON, B.Sc., N.D.A.

*(Scientific Department, Messrs David Bell, Ltd., Leith.)*

(With 7 Graphs and 2 Text-figures.)

A LARGE proportion of the "seeds" used in agriculture is included in the Natural Order Gramineae and, arising out of the present day insistence on a viability test previous to sowing, the laboratory germination of this class is a problem of importance.

Regarded from the point of view of volume of trade, the genus *Poa* is not of prime importance. Regarded from the point of view of the seed analyst, however, the fickle nature of the species of the genus in germination gives them considerable interest.

The present study was planned, in the first place, to analyse the action of the various factors bearing on germination and correlate them one to the other. The action of various salts was studied, and later, the soil as a substrate was introduced.

Numerous workers have shown that alternation of temperature and exposure to light are aids to germinating "seeds" of the Poas. These two aids are part of the method standard in British laboratories (Saunders<sup>(21)</sup>). In American laboratories a 1 per cent. solution of potassium nitrate is commonly used in place of tap-water as a source of moisture. The nitrate increases the speed of germination and the total number of seeds germinating.

*Material.* Samples of "seed" of the four common species (*compressa*, *memoralis*, *pratensis* and *trivialis*), crop 1924, were obtained and kept under room conditions until February, 1925, when tests were commenced. The purity of the samples to specific character was checked from Hellbo<sup>(7)</sup>. As each test was sown the "seed" to be used was drawn from the sample, care being taken that each individual was made up of a pair of "glumes" and a caryopsis.

*Apparatus and Methods.* Large, locally made, gas-heated tanks of the Jacobsen pattern, as shown in Fig. 1, were employed. Each 100 of "seeds" was sown on a circular blotting paper pad laid on two blotting



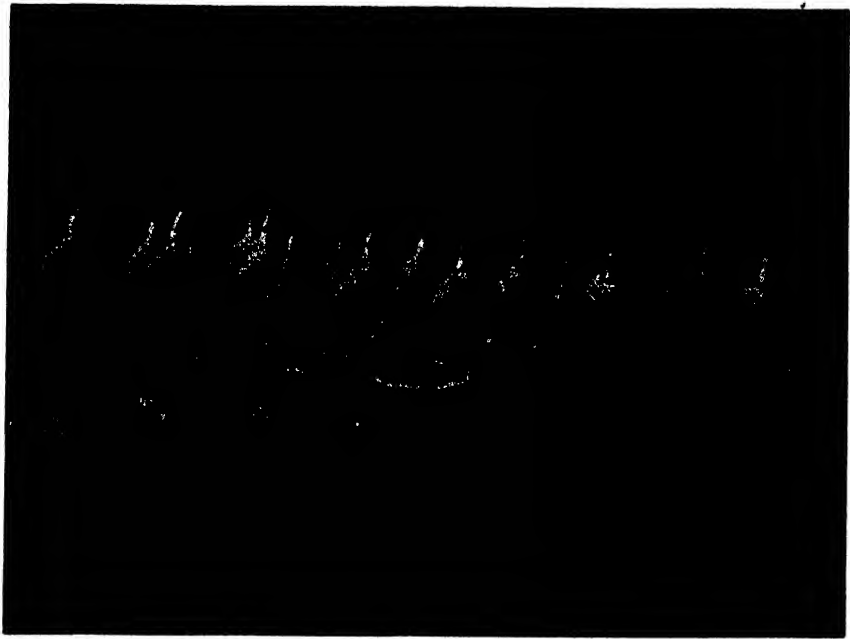


Fig. 1. Tests on germinating tank.

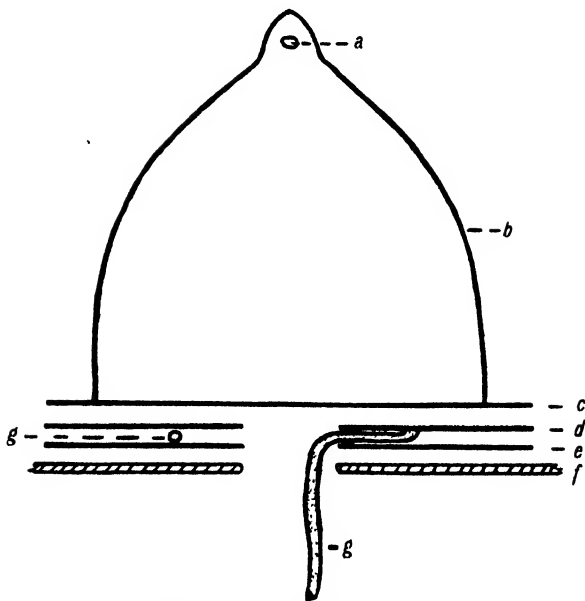


Fig. 2. Vertical section of germinating system.

*a*, Ventilating hole. *b*, Glass bell-jar. *c*, Blotting-paper "seed-bed" or pad. *d* and *e*, Blotting-paper rings. *f*, Glass bar supporting tests. *g*, Miners' wick.

paper rings. The pads were supported on glass bars running across the tank and kept moist by a length of miners' wick dipping into the tank water. Small clear-glass bell-jars covering the tests prevented excessive evaporation without excluding light (see Fig. 2).

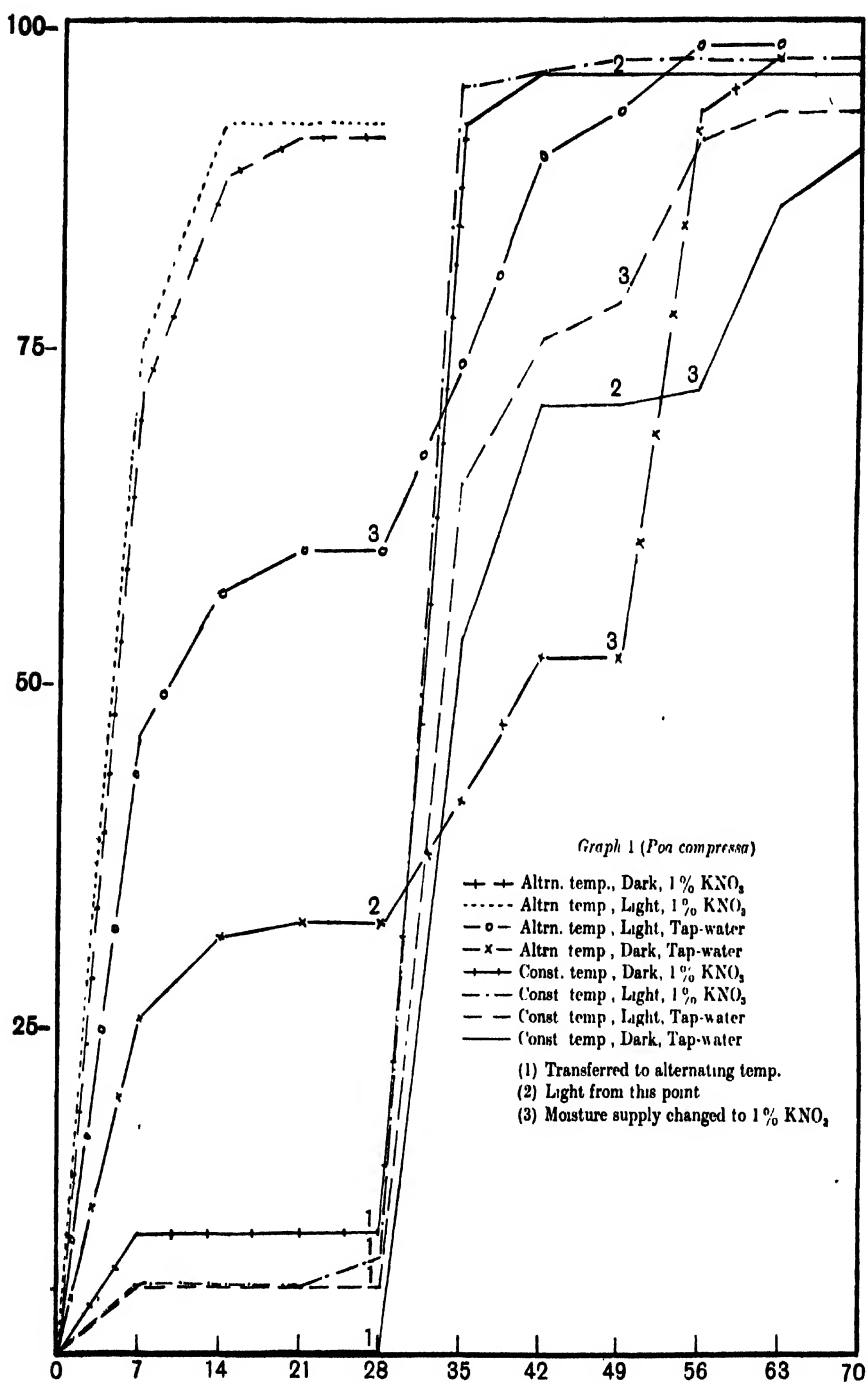
"Constant temperature" indicates that the water in the tank was kept practically steady at 25° C. "Alternating temperature" indicates that the tank water temperature was for 16 hours (5 p.m. to 9 a.m.) at 20°-23° C. and 8 hours at 35°-37° C. The temperature of the seed-bed would be a little lower than the water temperature, the gap depending on the difference between the water temperature and the temperature of the room. Harrington<sup>(6)</sup> has shown, however, that the alternation is more important than the temperature.

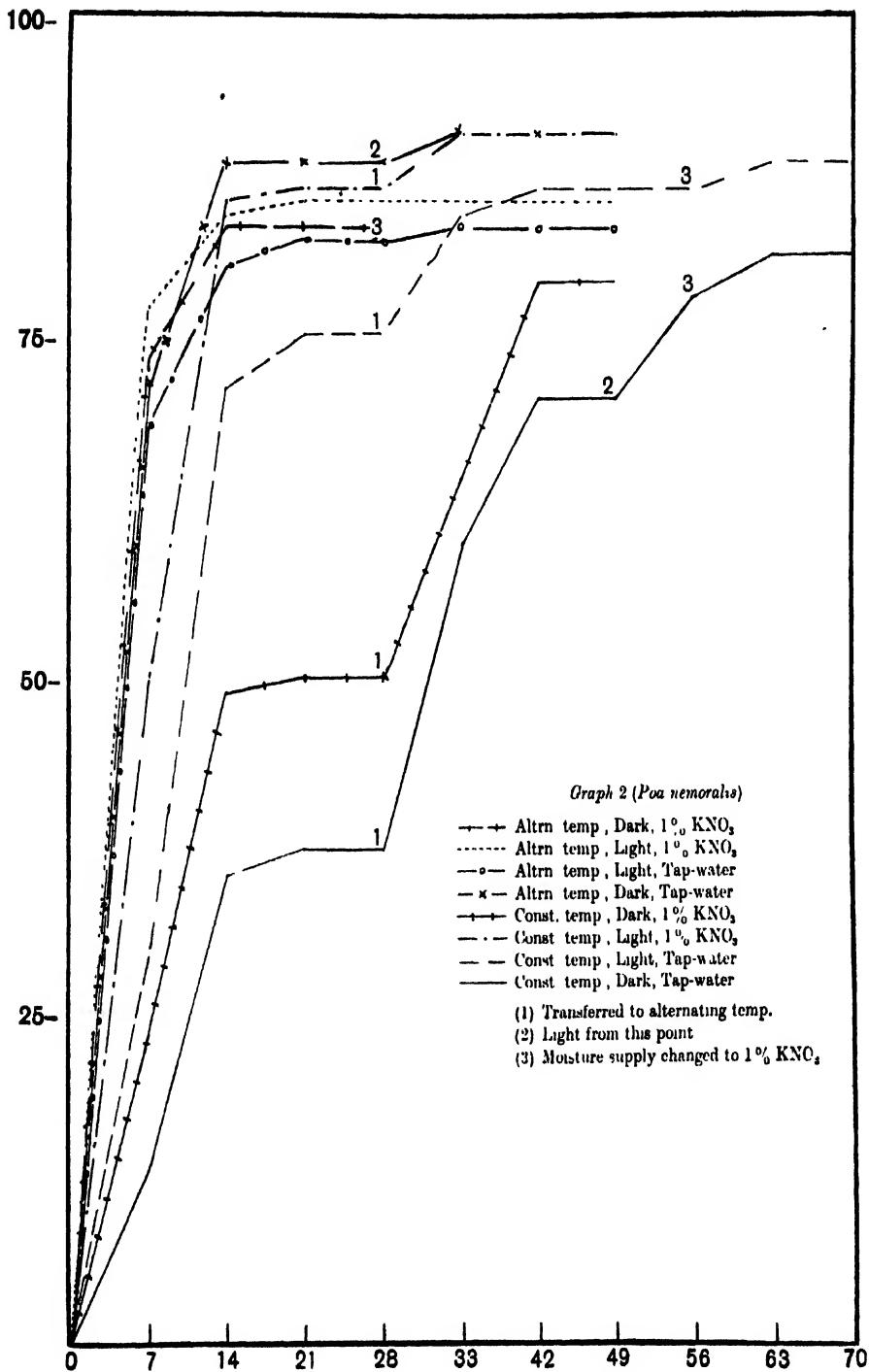
Tests "in the dark" were carried out by substituting brown bell-jars covered with brown paper for the clear-glass ones described above. When any salt solution was to be employed, the pad, rings and wick were moistened with it and the wick allowed to dip into a small jarful resting in the water on the tank bottom. Thus, except when the temperature conditions had to be varied, all tests were situated very close together and received exactly the same conditions except when these were deliberately altered.

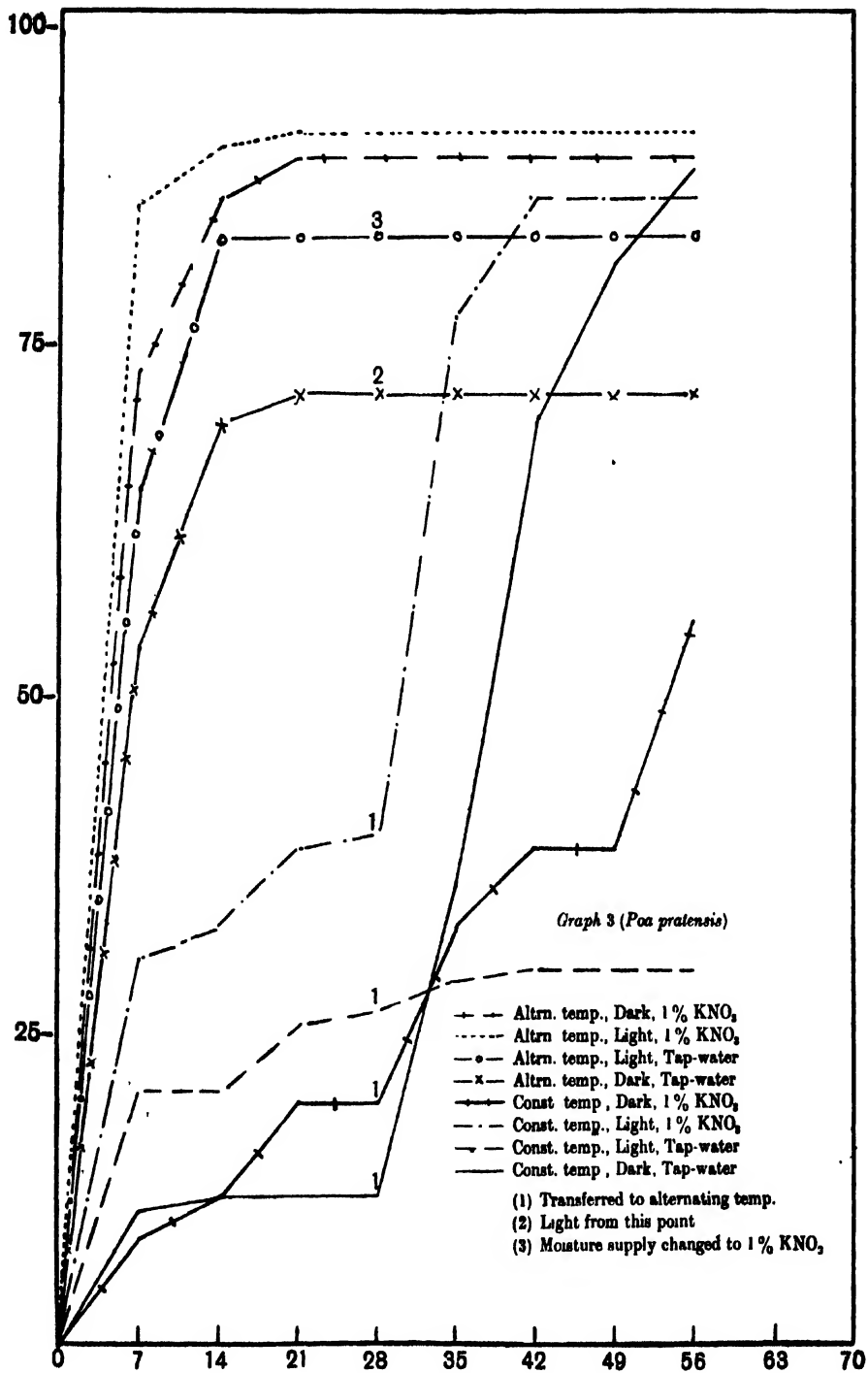
In all the graphs in this paper the period of the test in days is plotted along the base, and the number of "seeds" germinating, on the vertical scale. Every seven days the "seeds" which had germinated were counted off, except in the soil tests where they were counted off from day to day. Each of the four species mentioned above was in turn subjected to test by each of the undernoted methods, a pad carrying 100 "seeds" being allocated to each method.

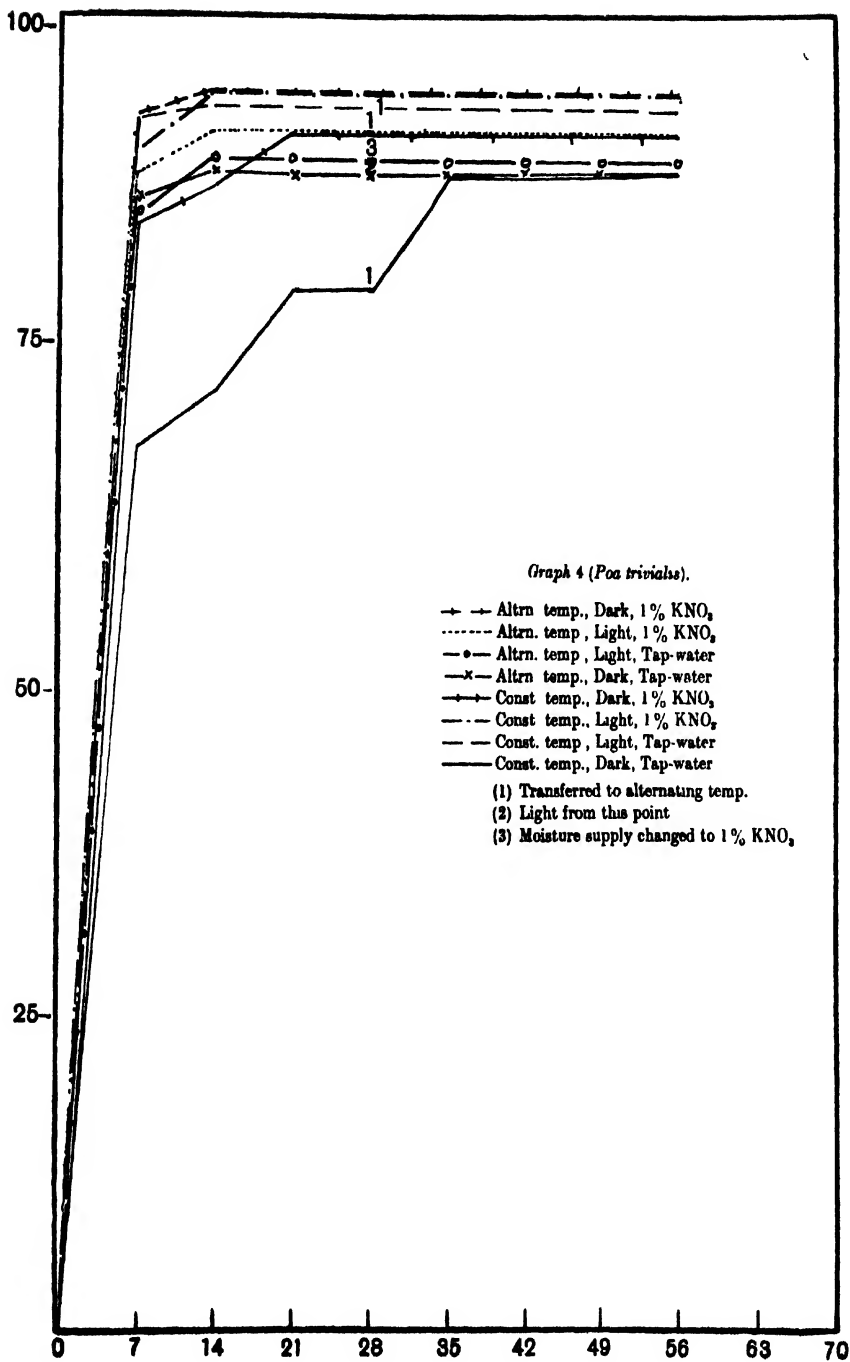
No.	Temperature	Lighting	Moisture supply
1	Constant temperature	Dark	Tap-water
2	" "	Daylight	" "
3	" "	" "	1 % solution KNO <sub>3</sub>
4	" "	Dark	" "
5	Alternating "	" "	Tap-water
6	" "	Daylight	" "
7	" "	" "	1 % solution KNO <sub>3</sub>
8	" "	Dark	" "

*Discussion of Graphs 1-4.* Considering for the moment Graph 1, which applies to *Poa compressa*, and dealing only with the first 28 days (the standard period for a routine test), it is seen that the curves form two main groups. The first group (methods 1-4) having been subjected









to constant temperature is of low germination. The second and higher group had alternating temperature. The difference between the two groups is distinct and conclusive. Within each group a certain arrangement manifests itself. At the base of each group lies the pad receiving no stimulation either from light or potassium nitrate. Next in ascending order comes the pad receiving light, then the pads receiving potassium nitrate. Potassium nitrate seems to be effective irrespective of the action of light.

When these tests had run their standard course (28 days) each pad was "stepped up" one place in what had thus been indicated to be the scale of stimulation. Pads 1-4 were now subjected to alternation of temperature: pads 4 and 5 were illuminated: pad 6 was changed to potassium nitrate solution, while pads 7 and 8 were withdrawn. The effect of these changes was immediate and striking, confirming the tentative conclusions based on the first period. The pads of the low group when transferred to alternating temperature responded at once, giving curves similar to their counterparts of the first 28 days, and differing only in that they rose higher—this might be explained by the pre-soaking. Following the curves of the "low group" through, it is seen that they tend to flatten out at various points, but that then the introduction of another "step up" in stimulation produces another rise. The two pads on the alternating temperature group behave in a similar sort of way: "seed" on pad 5 when illuminated at the 28th day immediately recommenced germination and continued until the 42nd day, when it stopped: given nitrate solution at the 49th day "complete germination" resulted. Pad 6, tested by the British standard method (alternation of temperature, light and tap-water), stopped germinating on the 21st day with a total of 60 per cent.: given potassium nitrate on the 28th day it eventually exceeded any other pad.

It is thus seen that *Poa compressa* is very sensitive to conditions of temperature, light and the character of the moisture supply. Alternation of the temperature is of first importance followed by the supply of potassium nitrate. These two together can produce satisfactory results and render the analyst independent of the light supply, but it is better to allow access of light rather than to screen the tests.

*Poa nemoralis* (Graph 2) appears to be less sensitive to the three factors studied than was *P. compressa*, and further, they seem capable of replacing each other to a greater extent. For instance the pads subjected to alternating temperature all germinated at or about the maximum and the differences between them are not significant.

Therefore, it would appear that alternation of temperature is the only essential factor with this species, light being practically unnecessary and tap-water sufficient. In the "constant temperature group," however, a certain arrangement manifests itself, in that the two pads in the dark (1 and 4) are significantly lower than the two in the light (2 and 3). Further, in either pair the pad receiving  $\text{KNO}_3$  is the higher of the two. "Stepping up" the pads produced significant responses, as was the case with *Poa compressa*. It may be concluded from these tests that *Poa nemoralis* will give satisfactory results when any two of the three stimulating factors are operative.

The results obtained with *Poa pratensis* (Graph 3) are somewhat similar to those obtained with *P. nemoralis*, though the effect of alternating temperature appears to be of greater importance and not replaceable by potassium nitrate and light. The behaviour of pad 2 (constant temperature, light and tap-water) is difficult to account for.

*Poa trivialis* is quite insensible to variations in the conditions as studied, except that the pad lowest in the "scale of stimulation" (constant temperature, dark and tap-water) is significantly lower than the others at the end of the standard period. Further, this deficit is made good by "stepping up," i.e. alternating the temperature.

The data offered here do not warrant any extended theoretical discussion of the processes underlying the various effects noted—especially apparent in the case of *Poa compressa*. Each factor evidently has a multiple effect, affecting more than one aspect of the life of the organism or, perhaps more accurately, the activities of the individual cell.

Harrington<sup>(6)</sup> in reviewing the subject of alternation of temperature mentions a number of hypotheses advanced by earlier workers to account for somewhat similar effects in other species. Briefly these are:

- (i) That temperature alternation affects water intake.
- (ii) The reserve of materials made available at any given temperature is wholly or largely used up in respiration, but a slight surplus becoming soluble at a higher temperature is available for growth when the temperature is lowered with consequent reduction of intensity of respiration.
- (iii) The whole effect of temperature alternation is due to oxygen relationships.

(iv) With alternation of temperature there is more lively gaseous interchange with consequent increase of respiration.

In discussing these with reference to the present study a number of lines of evidence taken from work covering the general flora may be



cited, though their application to germination must be guarded. Hoagland and Davis<sup>(8)</sup> discussing the absorption of ions by plants from dilute solutions show that light exerts a considerable effect in the case of an alga (*Nitella*). These writers conclude that the absorption of ions by plants from dilute solutions involves energy exchanges, with light as the ultimate source of energy, and they suggest that this absorption is intimately related to growth and metabolism. Jeffs<sup>(10)</sup> suggested that the light-growth-effect reaction of multicellular organs is not due to increase in cell elongation simply, but to some other effect of light such as change in the rate of cell division. The periodic nature of cell division noted by Kellicott<sup>(12)</sup> in *Allium cepa*, which probably holds good for many other plants, may be correlated in some way with the germination response to periodic alternation of external conditions and in this connection it is interesting to note that Reed<sup>(18)</sup> has shown that *Spirogyra* can be stimulated to divide by keeping the filaments at a low temperature in the dark and then transferring to optimum conditions of light and temperature.

That germination in its initial stages, at least, is not growth simply, must be remembered; and, as is well known, conditions affecting growth do not necessarily affect germination with equivalent force but the possible energy effects of light and temperature and their effect on cell division are clearly indicated. Other effects, such as increased permeability, quickened gaseous interchange and altered respiration likewise probably add their quota to the germination responses noted above.

In this discussion and also in what follows it is considered unnecessary to allow for any effect of the plant on the culture medium as reported by Hoagland<sup>(9)</sup> because of the short period during which the plant is active: growth has proceeded only a short way when the seedlings are counted off.

Turning to consideration of the stimulation resulting from the use of potassium nitrate it would again appear that the effect is complex. Most of the published work connected with this aspect deals with tissues from the mature plant or at least with the plant long after germination.

Nobbe, Shröder and Erdman<sup>(16)</sup> indicate that potassium has an effect on the translocation of starch. Copeland<sup>(2)</sup> states that potassium is a direct factor in increasing the turgor of the cell. Purvis<sup>(17)</sup>, working with *Dactylis*, concludes that potassium salts influence the physiological condition of the plant or its chemical composition. Macallum<sup>(13)</sup> has shown that potassium is always found where new outgrowths are in

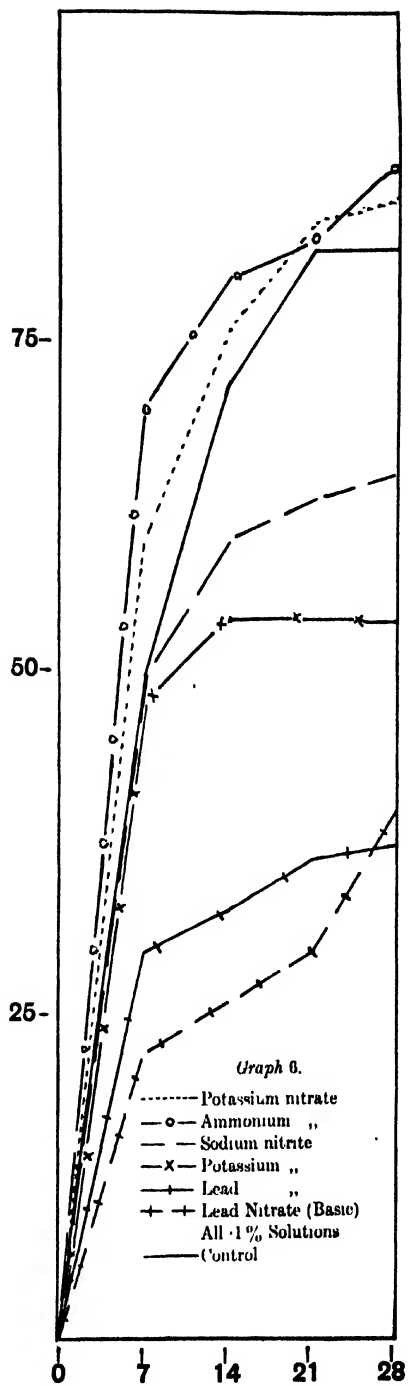
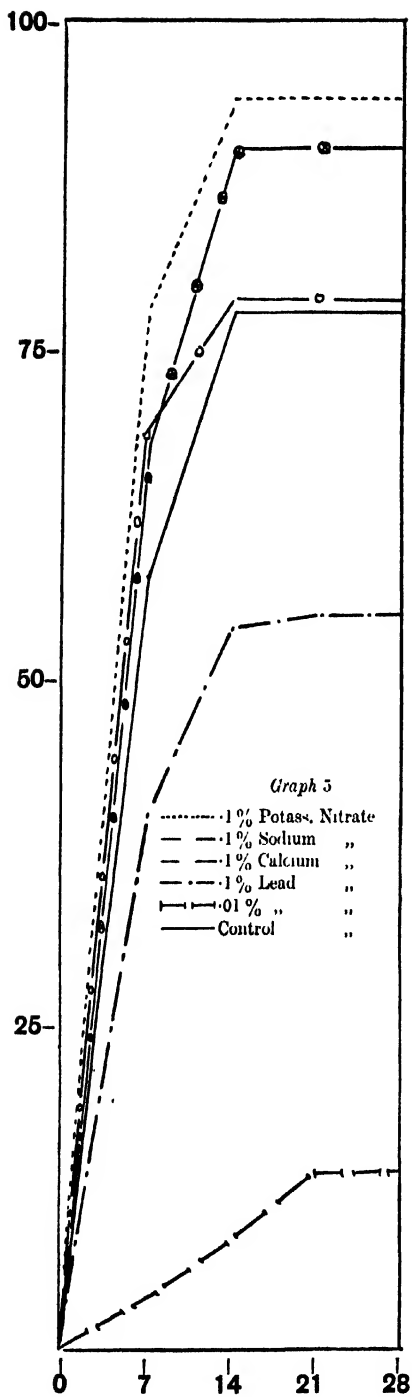
process of formation, while Dowding<sup>(4)</sup> concludes that potassium is essential to all meristematic tissue. Reed<sup>(18)</sup> has shown that potassium is necessary for the germination of moss spores. This general and widespread connection between potassium and actively developing tissue, coupled with the fact that Reed<sup>(18)</sup> found potassium essential for mitotic division, leads to the conclusion that potassium affects the dividing nucleus in a favourable way. Another mode of action for potassium may be to stimulate respiration as noted by Matthews<sup>(14)</sup> who also says, "The general richness of potassium in cells of widely different character indicates that this element must be concerned with some fundamental process or condition in the cell, and it is possible that that process is respiration. But just why it is favourable and what its real function is it is impossible to state."

To sum up, it seems reasonable to believe that each of these three factors studied has a number of modes of action, the value of these depending on the species in question and the condition of the seed at the time of the test. Their relation to the question of increased or accelerated nuclear division, though not previously considered in connection with germination effects, is undoubtedly of some considerable significance.

#### FURTHER WORK IN THE LABORATORY.

The next step was to compare the action of various salts in solution when used as the moisture supply. All this work was done on the sample of *Poa compressa* used for the tests shown on Graph 1. The salts selected included the nitrates commonly used as manures in general agriculture, *i.e.* calcium and sodium nitrates. Lead nitrate which Berry<sup>(1)</sup> found stimulating to the growth of oat *plants* was also included. All the tests shown on either Graph 5 or 6 were run simultaneously.

For Graph 5, 0.1 per cent. solutions (except one pad of lead nitrate) were employed and the results indicate that in promoting germination nitrates of potassium, sodium and calcium are superior to the control (tap-water) in the order named. Lead nitrate proved to be distinctly depressant, though of the two concentrations used the lower one had less effect. It was very interesting to note in this and all other tests with lead nitrate in concentrations such as these, that the glumes of the "seeds" all expanded and the appearance of the test at an early stage promised a high result. The concentrations used are very much higher than those employed by Berry. Later tests (when the seed may have altered in character) with a graduated series of concentrations in



light and dark indicated that at no strength was a solution of lead nitrate stimulating to germination.

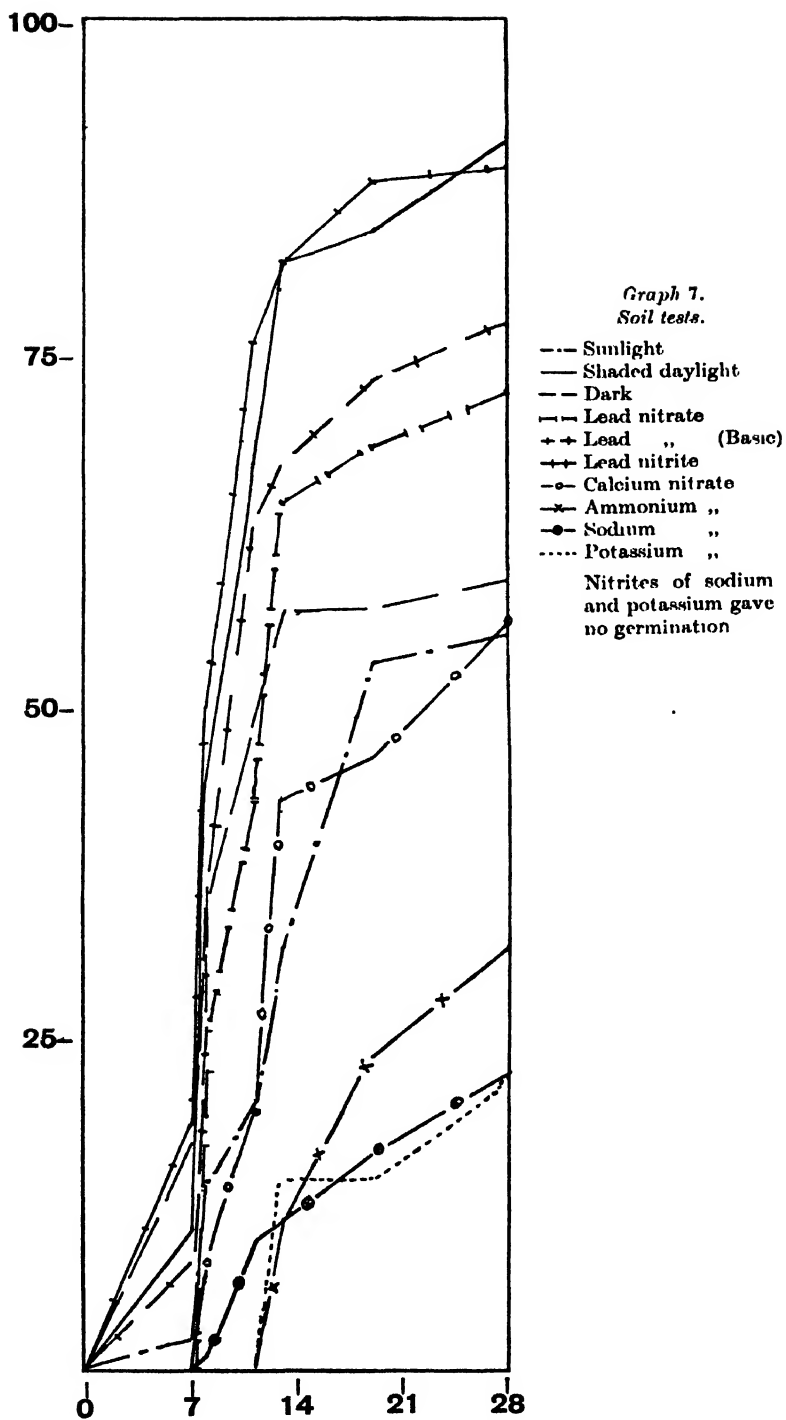
In Graph 6, again, a number of salts are compared. Ammonium nitrate was found to be at least equal to potassium nitrate in stimulating effect. Sodium and potassium nitrites were depressant while the lead salts, nitrite and basic nitrate, were also extremely depressant. The relative positions of sodium and potassium nitrites are interesting as they are the reverse of the nitrates of these bases, and suggest that the ions must not be considered alone but together, though the different effect of the different products following on ionisation must not be forgotten. Stiles<sup>(22)</sup> and a number of observers mentioned by him have shown that potassium is more rapidly absorbed by plant tissue than sodium, and either of them more rapidly than calcium: this is the order in which the nitrates of these bases fall when arranged according to their stimulative effect on the germination of *Poa compressa*. The effect of lead salts in proportion to their concentration is in agreement with the findings of Dilling<sup>(3)</sup> who noted the effect of lead acetate on cress and mustard seedlings.

#### SOIL TESTS.

The same sample of *Poa compressa* was used for the soil tests as was employed in the laboratory tests.

*Method.* Small pots (60's) were filled with some 150 gm. of air-dried potting soil passed over a No. 6 sieve. 0.1 gm. of salt was dissolved in 100 c.c. of tap-water and this used to saturate the soil, one salt being allocated to each pot. The surface of the soil after mixing was lightly pressed level and the seed sown one by one, each being gently pressed into the surface. Williams<sup>(23)</sup> has shown that for *Poa pratensis*, at least, the seed should not be buried.

In the tests shown on Graph 7, in addition to the pots used for the seven salts experimented with, three pots were included which had been moistened with tap-water only—these were included to try the effect of different degrees of lighting. Each pot was covered with a small square of 21 oz. glass and over all a sheet of "glassene" paper was spread, except over one of the tap-water pots. The seed in this pot was thus exposed to the full glare of the sun, the curve of this test being designated "sunlight." One of the remaining tap-water pots had its glass cover painted over with black enamel: this pot provided the "dark" results. The tests were conducted in a greenhouse in the south-east of England, the sunlight being brilliant and the temperature as



read on the maximum-minimum thermometer very much like the alternation produced in the laboratory. "Seeds" germinating were counted off from day to day and the tests ran for 28 days. As the soil in the pots dried it was moistened with its appropriate solution by sub-irrigation. It is of interest to note that the "dark" pot and the pots moistened with nitrites (all bases) did not dry so quickly as the others, this difference between the pots being very striking.

Consideration of the results plotted in Graph 7 shows that of the three pots moistened with tap-water the one in shaded daylight is very significantly higher than those in "dark" or "sunlight." This indicates that the pots moistened with salt solutions were in the best light conditions.

Morada<sup>(15)</sup> has shown that exposure to full sunlight for the whole period available per day has a depressing effect on the germination of *Carica papaya*, though exposure for a short time proved beneficial. Dark, he found, induced dormancy.

Considering the tests where salt solutions were employed, the very striking fact emerges that the salts found to be stimulating in laboratory water culture proved to be the most depressant in the soil, while the salt most depressant in water culture was the only one which equalled or exceeded the best control (tap-water). As has been noted, all the nitrite pots held the moisture much better than the others, but though the lead nitrite pot produced the largest number of plantlets the nitrites of sodium and potassium produced not one. Another point of interest was that the surface of the soil in all the pots excepting the "dark" and the "sunlight" pots and those moistened by sodium and potassium nitrite was covered in varying degree with a green alga. The lead nitrite pot had easily the heaviest growth of alga. Thus, while it may be that some of the stimulating effect of the lead nitrite on the seed was due to the better moisture conditions, it must be noted that the dark pot and the sodium and potassium nitrite pots provided equally good physical conditions but very much lower biological results.

Why there should be such a complete reversal of germination effect between the two groups of salts is difficult to explain, but the results certainly confirm the conclusion of Jensen<sup>(11)</sup> who worked with wheat seedlings. Discussing the toxic effect of salts, that writer concludes that it does not follow that because a salt is highly toxic in solution it is equally so in the soil, nor that one which holds a relatively high position in the toxic table of the soil should occupy the same relative position in solution cultures. It would appear too, from the results here

presented, that the same sort of conclusion is equally true of the stimulating effect.

Berry<sup>(1)</sup> noted in growth trials with oats that lead salts induced fleshier, broader and darker coloured leafage: this too was clearly seen in the tests reported here, the first leaves of the seeds which germinated on the lead pots being decidedly darker in colour, broader and more fleshy than those on the non-lead pots.

#### GENERAL DISCUSSION.

All the results reported here bear on several practical problems, more particularly on such questions as to how far laboratory tests are referable to soil conditions, and also in what way artificial manures may affect germination in the field. With regard to the first question it is interesting to note that the soil test in "shaded daylight" compares favourably with any laboratory test, though it must be remembered this pot would be under ideal conditions not likely to occur in the open field. With regard to the second question it is indicated that none of the manurial salts commonly employed in agriculture should be applied while grass "seed" is germinating. While the average concentration in the field would never be as high as those used in these tests, owing to the irregular distribution of the manure, certain areas would undoubtedly reach the concentrations here described and so, possibly, lead to a "patchy braid." These results obtained with *Poa compressa* may not apply absolutely to other grasses more commonly employed in agriculture.

The effect of lead salts on the growth of the alga and the physiological effect evidenced by the deep coloration accompanied by increased fleshiness of the grass leaf requires further study but of peculiar interest are the results obtained by Reznikoff<sup>(19)</sup> working with *Amoeba*. This worker using  $PbCl_2$  found two effects, one on the cell surface he ascribed to an effect of the acid formed following on ionisation of the salt. A second and more fundamental effect occurring within the protoplasm he ascribed to the cation. Only the first mentioned of these two effects was lethal. Reznikoff suggests that lead within the cell reacts with the phosphates and carbonates. This may be the mechanism resulting in the leaf effect described above.

## SUMMARY.

The paper in its first part deals with the germination response, in the laboratory, of four species of *Poa* to variations of temperature, light and the nature of the moisture supply. The second portion deals with the same response of *P. compressa* to solutions of various salts used as moisture supply. The third part deals with the germination of *P. compressa* in the soil when all the above factors were varied. In the laboratory, alternation of temperature proved to be, generally, the most efficient stimulant followed by light and a solution of potassium nitrate as moisture supply. *P. compressa* proved to be more sensitive than any of the other species considered, *P. pratensis* and *P. nemoralis* rather less so, while *P. trivialis* was almost insensible to variations of the conditions. In the soil, excess of light (sunlight) proved to be depressant. Some salts, e.g. potassium nitrate, sodium nitrate, ammonium nitrate, etc., proved to be stimulating in solution culture, while salts of lead were depressant. As a general statement these salts, found to be stimulating in solution culture, proved to be more depressant in soil than those found depressant in the solution. Lead salts in the soil produced broader, deeper coloured leafage than the controls. Nitrites had a striking physical effect in preventing the drying up of the soil.

## LITERATURE.

- (1) BERRY, R. A. (1924). The Manurial Properties of Lead Nitrate. *Journ. Agric. Sci.* xvi, Pt. 1.
- (2) COPELAND, E. B. (1897). The Relation of Nutrient Salts to Turgor. *Bot. Gaz.* xxiv.
- (3) DILLING, W. J. (1920). Influence of Lead and the Metallic Ions of Copper, Zinc, Thorium, Beryllium and Thallium on the Germination of Seeds. *Ann. App. Biol.* xiii, No. 2.
- (4) DOWDING, E. S. (1925). The Regional and Seasonal Distribution of Potassium in the Plant Tissues. *Ann. Bot.* xxxix.
- (5) GUTHRIE, F. B. and HELMS, R. (1905). Pot Experiments to Determine the Limits of Endurance of Different Farm Crops to Certain Injurious Substances. *Agric. Gaz., New South Wales*, xvi.
- (6) HARRINGTON, G. T. (1923). The Use of Alternating Temperature in the Germination of Seeds. *Journ. Agric. Res.* xxiii, No. 5.
- (7) HELLBO, E. (1924). Some Criteria by means of which Seeds of the *Poa* Species can be determined. *Tidskrift for Planteavl.* xxx.
- (8) HOAGLAND, D. R. and DAVIS, A. R. (1923). Further Experiments on the Absorption of Ions by Plants, including Observations on the Effect of Light. *Journ. Gen. Phys.* vi, No. 1.
- (9) HOAGLAND, D. R. (1923). The Effect of the Plant on the Reaction of the Culture Medium. *University of California Technical Paper No. 12 of the Agric. Exper. Stn.*



- (10) JEFFS, R. E. (1925). The Elongation of Root Hairs as Affected by Light and Temperature. *Am. Journ. Bot.* XII, No. 9.
- (11) JENSEN, G. (1907). Toxic Limits and Stimulation Effects of some Salts and Poisons on Wheat. *Bot. Gaz.* XLIII.
- (12) KELLICOTT, W. E. (1904). The Daily Periodicity of Cell Division and of Elongation in the Root of *Allium*. *Bull. Torrey Bot. Club*, XXXI.
- (13) MACALLUM, A. B. (1905). On the Distribution of Potassium in Animal and Vegetable Cells. *Journ. Phys.* XXXII.
- (14) MATTHEWS, A. P. (1924). Some General Aspects of the Chemistry of Cells. In *General Cytology*. Chicago.
- (15) MORADA, E. K. (1924). The Effect of Sunlight on the Germination of *Papaya* Seed. *Philippine Agric. Rev.* XVII, No. 1.
- (16) NOBBE, F., SCHRÖDER, J. and ERDMAN, R. (1870). Über die organische Leistung des Kalium in der Pflanze. *Landw. Versuchs-Stat.* XIII.
- (17) PURVIS, D. N. (1918). The Effects of Potassium Salts on the Anatomy of *Dactylis glomerata*. *Journ. Agric. Sci.* IX, No. 4.
- (18) REED, H. S. (1907). The Value of Certain Nutritive Elements to the Plant Cell. *Ann. Bot.* XXI.
- (19) REZNIKOFF, P. (1926). Micurgical Studies in Cell Physiology. *Journ. Gen. Phys.* x, No. 1.
- (20) RUSSELL, E. J. (1921). *Soil Conditions and Plant Growth*. 4th ed. London.
- (21) SAUNDERS, C. B. (1923). *Methods of Seed Analysis*. Cambridge.
- (22) STILES, W. (1924). *Studies in Permeability*. London.
- (23) WILLIAMS, R. D. (1922). Depth of Sowing Grass and Clover Seed. *Journ. Min. Agric.* XXIV, Nos. 1 and 2.

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## THE TRANSMISSION OF "MOSAIC" DISEASE IN HOPS BY MEANS OF GRAFTING

BY T. C. THRUPP, B.Sc., A.R.C.Sc.

(With Plate XI.)

### INTRODUCTORY.

THE characteristic symptoms of the "mosaic" disease of hops are, mottled green and yellow areas in the leaves (called "mosaic" mottling), the curling in of the edges (recurved), and a noticeable brittleness of the leaves and petioles and of the tips of the bines. The yellow areas are translucent and are clearly visible when a white paper sheet is placed a few inches behind the leaves, or when these are viewed against the sky. The earliest symptoms of an attack are "mosaic" mottling and abnormal shape of the newer leaves of some of the laterals, while the bines themselves may have grown to their full length, produced hops, and have apparently quite normal leaves. In the following season, in addition to all the characteristic symptoms of the disease, the bines fail to grow to their normal length, fall away from the strings or the poles, die back at their tips, and produce few or no hops. In the final stage of the disease, in the same or the following season, the whole plant dies. Occasionally one hop "hill," originally planted with one hop "set<sup>1</sup>," produces both "mosiac" and healthy bines. "Mosaic" disease occurs in many hop gardens in this country and frequently causes serious losses; it necessitates prompt grubbing of infected "hills" to prevent its rapid spread. An account of the disease from the economic point of view has been given by Prof. E. S. Salmon(2).

As no evidence has been produced to show that any visible organism is associated with the disease, it has been generally assumed that the cause must be a "virus." Miss Lacey(1) has shown that protozoa are not present in the phloem of affected hops. Preliminary experiments were carried out at the South-Eastern Agricultural College, Wye, in which the expressed sap of diseased hops was injected into healthy hops(5)

<sup>1</sup> In commercial practice more than one "set" or cutting is frequently planted to the "hill."

but no positive results were obtained. In the case of certain "virus" diseases of plants, grafting has been found a necessary means of investigation; this method was practised in 1925 by Salmon and Ware<sup>(5)</sup>, with the primary object of determining whether a tender and succulent plant such as the hop would submit to such treatment. It was shown that grafting as a process was feasible, but no work with "mosaic" affected material was carried out. From April 1926 and onwards to July grafting was used in the investigations described in the present paper, both for determining whether the disease could be transmitted by grafting, and in order to investigate a further and subsidiary problem which had arisen as far back as 1922. In that year, reports had been received from hop growers (see (1), p. 12) that a new variety of hop—"M 45"—bred at Wye by Prof. Salmon, which had never shown symptoms of "mosaic" disease, appeared to be infecting neighbouring hops of commercial varieties and might, therefore, prove to be a "carrier."

#### EXPERIMENTAL.

Material was available at Wye, both in the experimental hop garden and in a stock of specially collected plants grown in pots in a greenhouse, and in the hop gardens at the East Malling Research Station. During a part of March and in April a number of trial grafts were made in the hop garden at Wye, in order to gain experience in the process of grafting. In this trial grafting and in the experiments themselves, the stock bine was cut across with a clean safety razor blade, and a V-notch cut in that part of it left on the plant. The selected tip was placed on a clean glass slip and cut to fit the notch. The wedge-shaped end of the tip was then inserted in the V-notch and bound in place with a thin rubber strip, the last turn being fastened with rubber solution. In some of the practice grafts, female scions were grafted on to male stocks; in one case a male scion was grafted on to a female stock; in several cases double grafts were made by grafting one female scion on to a short piece of bine from another female plant, while this again was grafted on to a male stock. In most cases, and also throughout the experiments, the graft was made about 5 cm. above the soil and the scions were about 15 cm. long. Being grafted early in the season the scions of trial grafts grew to the top of the wire work (about 14 ft. from the ground) and in due course produced a normal crop of hops. The male scion also grew well and flowered. In one case a lateral from the middle section of a double graft grew to the top of the wire work and also produced a good crop of hops. This preliminary work showed that grafted healthy hops

will grow as well as normal ungrafted bines, provided the grafting is carried out sufficiently early in the season.

When using healthy material 87.5 per cent. of grafts succeeded, with diseased stocks the percentage successful was only 14.3. The grafting experiments, which began at the end of May, are classified below according to the problems in view.

*Upward passage of the supposed virus from "mosaic" stock.* Ninety-four healthy susceptible tips were grafted on to "mosaic" stocks. Of these grafts 16 "took" and grew. The scions were as follows: (a) commercial varieties of hops known to be susceptible to the disease, viz. Tutsham (7), Cooper's White (2), Eastwell Golding (1), Farnham Whitebine (1), Cobb's Golding (1); (b) male hops (2); (c) the seedling variety M 45 (2). All the scions of the commercial varieties (with the exception of the Farnham Whitebine<sup>1</sup>) developed "mosaic" symptoms. In view of its known history, it was not expected that the two scions of M 45 would develop the "mosaic" symptoms. It was, however, surprising to find that one of these thrived to such an extent that it grew to a height of about 4.5 metres and remained free from all symptoms of the disease, while the remaining bines of the "mosaic" stock were nearly all dead from the severity of the attack. The two male hop scions grew and showed no "mosaic" symptoms, but in these cases the stock was not as badly attacked by the disease. One of these male hops was a plant growing at the Research Station, East Malling (hill 12 in the row of the Tutsham variety); the other was a "wild" hop (Ref. No. A). It is possible that these male hops may be immune; Prof. Salmon has reported<sup>(3)</sup> the existence of male hops immune to the "mosaic" disease.

A typical case of a susceptible scion on a "mosaic" stock may be given here. A Tutsham scion was grafted on to a "mosaic" stock on June 20th. All the shoots of the stock were affected with true "mosaic" disease. The grafted bine grew to about one metre in length without exhibiting any noticeable symptoms of the disease, but on August 3rd some of the primary leaves showed "mosaic" mottling. On August 16th

<sup>1</sup> The Farnham Whitebine scion lost its growing point by accident, and the lateral which took its place grew very slowly. In consequence the bine was only about 0.75 metre long by September 11th. Among the grafts made out of doors, it was found that the checks in growth were correlated with less intense "mosaic" symptoms when these did develop. Further, it was noticed in several cases that if the symptoms did not appear until September they were less definite than usually observed. In the case of this Farnham Whitebine scion, the stock had both "mosaic" and healthy bines at the time of grafting, and the healthy bines remained free from "mosaic" symptoms to the end of the season. In view of the above facts, the negative result obtained must not be unduly stressed.

the mottling was much more pronounced and most of the leaves curled downwards. By the end of August all primary leaves showed the characteristic symptoms of the disease, and the bine was indistinguishable in appearance from the "mosaic" bines of the stock. The grafted bine had grown to a length of 1.25 metres by the end of the season. The scion was obtained from a healthy plant growing in a pot, the remaining shoots of which were free from the disease throughout the season.

*Upward passage of the supposed virus from "carrier" stock.* Forty-four grafts were made, using the variety M 45 as stock. Of the 26 grafts which "took," the scions were of the following varieties: Tutsham (7), Eastwell Golding (5), wild hop "C" (3), Farnham Whitebine (2), wild hop "B" (5), wild hop "D" (1), wild hop "E" (1), and 2 male hops. The first 17 cases developed "mosaic" symptoms, while the other 9 cases did not do so. It was noticeable that grafts made in the greenhouse on "carrier" stock did not develop the curling of the leaf edges in the same manner; generally speaking the curling in was on the upper surface, but not in so marked a manner as the curling in on the lower surface observed with the outdoor grafts. The "mosaic" mottling was quite distinct. The highest temperature recorded in the greenhouse was 33° C.

The details of a typical case when using a susceptible scion may be given. A Tutsham tip was grafted on to an M 45 stock in the hop garden at Wye on April 19th. This scion grew and appeared normal and healthy until June 19th, when the primary leaves were seen to have "mosaic" mottling and their edges to be slightly curled downwards; the bine (graft) was then about 1 metre long. On August 20th it had grown to 1.79 metres, the primary leaves were much more mottled and the curling was more pronounced. Subsequently the leaves of the laterals grew out and all developed "mosaic" mottling with a certain amount of curling, those at the tips having abnormal outlines. This bine is lettered C in the photograph reproduced in the Plate.

*Controls.* As regards controls, all the parent hop plants, with one exception<sup>1</sup>, from which tips had been taken to graft on to "mosaic" and "carrier" stock, remained free from all symptoms of the disease throughout the season. As additional controls 24 single grafts of healthy susceptible scions were made on healthy susceptible stocks, and 7 double grafts were made from similar material. Of the single grafts 21 "took,"

<sup>1</sup> The experiment in which this control plant was used was discarded and is not included in the number 91, given in Table I.

the hops used being: Tutsham (9), Eastwell Golding (3), wild hop "B" (2), wild hop "C" (3), wild hop "D" (1), and wild hop "E" (3); in each case the stock used was of the same variety as the scion; all grew well and the majority produced hops. All remained free from all "mosaic" symptoms. Of the 7 double grafts, which were made in the manner already described for the practice grafting (*but* with all the component parts of the same variety), 6 were successful: Tutsham (2), wild hop "B" (2), wild hop "C" (1), and Eastwell Golding (1); all grew fairly well and none showed "mosaic" symptoms at any time. Similarly 5 "carrier" scions were grafted on to "carrier" stock, without inducing any "mosaic" symptoms to appear; two even reached a height of 14 ft. and produced a good crop of hops.

No special precautions were taken against insects during these experiments, because it was considered that grafting would give decided results in a very short time and because adequate controls were provided by leaving as many vines as possible on the hill which served as stock, and by keeping under observation all parent plants from which any scions were taken. A summary of the grafting experiments is given in Table I.

Table I.

*Summary of "mosaic," healthy and "carrier" grafting experiments.*

Stocks	Totals	Scions			
		Number of healthy		Positive result	Negative result
		Taken	Failed		
"Mosaic"	91*	13	78	10	3†
Healthy	24	21	3	0	21
"Carrier"	44	26	18	17	9‡
Number of "carrier"					
"Mosaic"	2	2	0	0	2§
Healthy	—	—	—	—	—
"Carrier"	5	5	0	0	5

\* See footnote on p. 178.

† Of these three scions which took, and from which negative results were obtained, two were from male hops which may perhaps be truly immune, and one was from the Farnham Whitevine which has already been discussed in detail (see p. 177).

‡ The origin of these nine scions was as follows: wild hop "B" (5), wild hop "D" (1), wild hop "E" (1), and the two male hops mentioned above at p. 178. The consistent negative results with respect to wild hop "B" may perhaps be regarded as evidence of immunity and the same may possibly be true also with respect to the others.

§ It was not expected that M 45 scions grafted on to "mosaic" stocks would give positive results (see above, p. 177).

The writer desires to express his indebtedness to Prof. E. S. Salmon, F.L.S., and to Mr W. M. Ware, M.Sc., for the benefit of their frequent advice given during these experiments.

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#### SUMMARY.

1. In these experiments, where susceptible scions have been grafted on to "mosaic" stock, all developed "mosaic" symptoms with one exception. Circumstances were not favourable in this particular case and it should be regarded as giving indecisive not negative evidence. From the other cases there is satisfactory proof that the supposed "virus" can be transmitted up the growing bine.

2. With the "carrier" experiments, all the known susceptible scions, which were grafted on to the "carrier" (M 45) stock, exhibited "mosaic" symptoms. The variety M 45 must, therefore, be regarded as capable of transmitting the disease to susceptible hops, and as being a "carrier."

3. There is no evidence that the process of grafting can of itself in any way induce "mosaic" symptoms to appear in healthy susceptible hops, or in the "carrier" M 45.

#### LITERATURE CITED.

- (1) LACEY, M. S. (1923). Protozoa and Virus Diseases of Plants. *Nature*, cxii, 2808, 280-281.
- (2) SALMON, E. S. (1923). The Mosaic Disease of the Hop. *Journ. Min. Agric.* xxix, 927.
- (3) — (1922). *Fifth Report on the Trial of New Varieties of Hops*. East Malling Research Station. (Ashford, Kent, July 1922.)
- (4) — (1923). *Sixth Report, etc.* (Ashford, Kent, July 1923.)
- (5) — and WARE, W. M. (1925). Virus Diseases and the Grafting of the Hop. *Gard. Chron.* lxxvii, 320-322.

#### EXPLANATION OF PLATE XI.

Four grafts made on "carrier" stock, photographed July 12th, 1926.

- A, Normal ungrafted M 45 bine, showing healthy foliage.
- B, Wild hop "B," grafted April 23rd, 1926, showing healthy foliage.
- C, Tutsham, grafted April 19th, 1926, showing down-curved "mosaic" affected leaves.
- D, Tutsham, grafted May 5th, 1926, showing well-marked "mosaic" symptoms.
- E, Eastwell Goldmg, grafted May 11th, 1926, showing weaker growth and evident but less pronounced "mosaic" symptoms.

0·175 nat. size.

(Received December 10th, 1926.)



THRUPP.- TRANSMISSION OF "MOSAIC" DISEASE IN HOPS BY MEANS OF GRAFTING (pp. 175—180).





# IMMUNITY OF POTATO VARIETIES FROM ATTACK BY THE WART DISEASE FUNGUS, *SYNCHYTRIUM* *ENDOBLOTICUM* (SCHILB.) PERC.

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(With Plates XII and XIII.)

It is a common horticultural practice to graft the shoot system of one plant on the root system of another. Since the grafted plant thus produced usually grows, it follows that the scion must be dependent on the stock for its mineral salts, and the stock on the scion for the substances necessary for its growth. Certain chemical compounds therefore must pass freely between stock and scion across the graft fusion layer.

These substances sometimes become changed after passing through this layer. Colin and Riolle<sup>(3)</sup>, Colin<sup>(4, 5)</sup>, and Colin and Franquet<sup>(6)</sup> proved that when the artichoke (*Helianthus tuberosus*) is grafted on the sunflower (*H. annuus*), the inulin, a carbohydrate which is characteristic of the artichoke, on passing into the sunflower, is rapidly hydrolysed and changed to saccharose.

Other compounds have been proved to remain unchanged after having traversed the fusion layer. Belladonna plants contain atropine and normal tomato and potato plants do not, but from the work of Laurent<sup>(15, 16)</sup> and Javillier<sup>(11)</sup> it is evident that when parts of Belladonna and tomato plants are grafted together, an alkaloid similar to, if not identical with, atropine is sometimes found in small quantities in the tomato tissues. Further, Javillier<sup>(11)</sup> grafted together portions of Belladonna and potato plants and found, both by chemical and physiological tests, that certain parts of the grafted potato tissue contained a substance apparently identical with atropine. Again, Grafe and Linsbauer<sup>(9)</sup>, on grafting together pieces of *Nicotiana tabacum* and *N. affinis*, found that the amount of nicotine in the *N. affinis* part became increased from nil,

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or practically nil (*N. affinis* used in their experiments contained no demonstrable quantities of nicotine), to as much as 1.67 per cent. on dry weight.

Brown<sup>(2)</sup> has shown that the mineral composition of the fruit of a given variety of apple varies according to the stock on which the variety is grafted. That grafting a scion on a stock may affect more than the mineral composition of that scion is suggested by the work of Barker and Grove<sup>(1)</sup>, who found that the acidity, and probably the tannin content, of apple juice is affected by the type of stock on which the apple is grafted.

Without going further into the literature of the highly controversial subject of the effect of stock on scion and of scion on stock, it is sufficiently clear, firstly, that a stock may cause a change in the mineral composition as well as in other properties of the scion grafted on it, such as acidity, essential oil content (Daniel and Ripert<sup>(8)</sup>), and probably tannin content; and secondly, that a shoot system may cause changes in the chemical composition of the stock on which it is grafted through the permeation of the tissues of the stock by substances built up in the scion.

Grafting, therefore, offers a method for determining whether a given plant character, such, for example, as immunity, is innate in the cell, or whether it is conditioned by substances which can travel freely about the plant; if the character is of the second kind the contributions of the various tissue systems, roots, foliage, etc., may be determined.

The present paper describes an attempt to investigate, by the method of grafting, the general nature of the immunity of certain varieties of potatoes from attack by the fungus causing Wart Disease, *Synchytrium endobioticum* (Schilb.) Perc.

For our present purpose the potato plant may be looked upon as being composed of three physiological units:

- (1) The root system, which supplies the plant with mineral salts and water.
- (2) The shoot system, the photosynthetic unit.
- (3) The tuber, or storage unit.

The problem resolves itself into building up, by grafting, composite plants of all possible types, and testing the tubers for any resulting effect in regard to immunity from, or susceptibility to, the disease. The following eight types are possible.

Reference will be made to these types both by number and by "formula."

Type	Root	Shoot	Tuber	Formula (Shoot / Tuber) (Root / Tuber)
1	I	S	I	$\frac{S}{I} / \frac{I}{I}$
2	S	I	S	$\frac{I}{S} / \frac{S}{S}$
3	S	I	I	$\frac{I}{S} / \frac{I}{I}$
4	I	S	S	$\frac{S}{I} / \frac{S}{S}$
5	S	S	I	$\frac{S}{S} / \frac{I}{I}$
6	I	I	S	$\frac{I}{I} / \frac{S}{S}$
7	S	S	S	$\frac{S}{S} / \frac{S}{S}$
8	I	I	I	$\frac{I}{I} / \frac{I}{I}$

S—susceptible, I—immune.

#### EXPERIMENTAL WORK.

Grafts of types 1 ( $\frac{S}{I} / \frac{I}{I}$ ), 2 ( $\frac{I}{S} / \frac{S}{S}$ ), 7 ( $\frac{S}{S} / \frac{S}{S}$ ) and 8 ( $\frac{I}{I} / \frac{I}{I}$ ) were dealt with in an earlier paper, Roach (17). In the experiments then reported it was found that shoots of susceptible and immune varieties respectively used as scions did not change the immunity or susceptibility of the tubers produced on the stocks.

In the experiments in question the effect of the grafted-on foliage was tested during the following season by growing the tubers produced by the grafted plant in contaminated soil. It might be objected that by this method any immunity-conferring or susceptibility-bestowing substance stored in the tuber would be "diluted" when the tuber gave rise to a new potato plant, and that the concentration of the substance might thus be insufficient to produce its normal effect. To meet this objection a few grafted plants were grown in contaminated soil, and the effect on the developing tuber was watched, instead of that on the plant arising from this tuber in the following season, as in the previous experiments. Again, however, no effect on the grafted-on foliage on the immunity or susceptibility of the tuber was detected. Confirmation of these results comes from the work of Köhler (14), who grew his grafted plants in contaminated soil, and sought the effect of grafting in the tubers as they developed on the actual grafted plant.

Grafts of types 3 ( $\frac{I}{S} / \frac{I}{I}$ ) and 4 ( $\frac{S}{I} / \frac{S}{S}$ ). To build up plants of these types in order to test the effect of a "foreign" root system on the

behaviour of the resulting tuber towards wart disease, grafts of the same kinds as those used for types 1 and 2 were first made. The soil in which the grafted plant was growing was washed away until the whole of the stalk was uncovered and exposed to light. A shallow trough was then built up around the stalk just above the level of the graft union and filled to a depth of 3 or 4 in. with dry sand. In this way the formation of tubers above the graft was induced, but root formation was prevented.

In experiment No. 519 Majestic (I)<sup>1</sup> was grafted as scion on Arran Chief (S)<sup>2</sup>. Three full-sized tubers were produced above the graft. All attempts to alter the behaviour towards wart disease of either the tubers or the leaves of the plants above the graft union failed. One of the tubers was tested by Glynne's method of infection by summer sporangia (10) and found to be immune.

The produce of four other grafted plants of these types was unfortunately lost through disease of various kinds during the 1924 season. In view of the successful building up of grafts of types 5 ( $\frac{S}{I}$ ) and 6 ( $\frac{I}{S}$ ) and of the results obtained from them, the repetition of the grafts of types 3 and 4 was postponed.

*Grafts of type 6* ( $\frac{I}{S}$ ). The problem to be solved by grafts of type 6 was whether an immune plant is able to bestow immunity on a susceptible tuber grafted on it and "fed" by it. In each experiment a cleft graft was made uniting the stolon of the grafted-on susceptible tuber to a conveniently sized stolon of the immune plant. It was found advantageous with all stolon grafts to inhibit normal tuber formation of the foster-plant somewhat by uncovering the portion of the stem below soil level and exposing it to light, leaving only the grafted stolon covered. In this way the development of new stolons was avoided. It was found that unless this precaution was taken, storage took place in existing tubers or in those arising on newly formed stolons, so that the grafted-on stolon, though remaining apparently quite healthy, did not increase in size. Normal produce from the foster plant could of course be obtained after the grafted-on portion had grown sufficiently, by merely covering a part of the stalk again. Covering the stalk at this late stage when the graft-union was complete did not prevent further increase in size of the grafted-on portion.

In experiment No. 613 (1) use was made of a very heavily warted piece of Arran Chief (S) tissue. An underground stem of this plant, which had turned upwards, had evidently become infected just as it

<sup>1</sup> I = immune.

<sup>2</sup> S = susceptible.

emerged from the soil and when its leaves were just beginning to expand. The whole leafy structure was heavily infected. It was grafted by means of its stem on a K. of K. (I) stolon.

The final condition of this graft is illustrated in Plate XII, fig. 1. The warted tissue can be seen in the bottom left-hand corner of the figure near the sixpence, which gives some idea of the size of the wart. The position of the graft-union is indicated by the binding threads just above the wart. Periodic examination with a lens showed that the actual number of sporangia was doubled during the period of the experiment; hence there was no suggestion of an inhibition of the disease as a result of the warted tissues being "fed" on food material derived from the immune plant. The healthy appearance of the union, the turgidity and the increase in size of the grafted-on part left no doubt that organic union had actually taken place: pieces of warted material similar to that used as the scion, but not grafted, became flaccid in less than a day.

In experiment No. 616 an Arran Chief tuber (S) (diameter about 1 cm.), having on it a wart about 1 mm. in diameter, was grafted by means of its stolon on a stolon of a K. of K. (I) plant. After about a week the wart commenced to grow, though the tuber did not increase appreciably in size. Successive stages in the development of the wart are shown in Plate XIII, figs. 5 and 6<sup>1</sup>. Here again there was no evidence of the immune plant having any inhibiting effect on the development of the wart. Examination of the junction showed that organic union had taken place, for the two vascular systems were joined.

In experiment No. 613 (2) the immune plant, again K. of K., was given an apparently still better opportunity to affect the grafted-on wart, for the latter was of microscopic size, and as far as could be seen from an examination with a binocular microscope, consisted of one summer sorus only. The graft was made on August 11th, 1923<sup>1</sup>. The photograph from which Plate XII, fig. 1 was reproduced was taken on August 16th. Plate XII, fig. 2, is an enlarged view taken on August 17th. Plate XII, figs. 3 and 4, show the condition of the wart on September 4th and 10th respectively. By suitable arrangement of the camera, etc., it was possible to take the last three photographs so that the magnification was the same each time. The diameter of the tuber remained at 0.5 cm.; but, as can be seen from the figures, the wart increased very greatly in size.

<sup>1</sup> Unfortunately no photograph was taken of these tubers at the time when the graft was made.

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The behaviour in the above three grafting experiments show that at least so far as the two varieties, Arran Chief (S) and K. of K. (I) are concerned, the immune host plant was unable to inhibit the growth of the wart on the grafted-on tissue, even though that tissue was entirely dependent on the immune plant for all the materials used by it in its growth. There is still the possibility that if the susceptible tissue had been grafted on the immune plant before the wart disease fungus had become established, then the immune plant by means of the products supplied by it to the grafted-on tissue, might have made infection by the parasite impossible. In experiment No. 528 a piece of stolon about 3 cm. long, free from wart disease, raised in uncontaminated soil, and not having yet begun to swell into a tuber, was cut from a Garland (S) plant (white-tubered), and grafted on near the end of a stolon—at that time devoid of tubers—of an Arran Victory (I) plant (purple-tubered). The graft was made on June 3rd, 1923. On July 10th the top soil was carefully washed away, when the grafted-on stolon was found to have increased greatly in length and bore three white tubers of small “seed” size, whilst the foster stolon now bore three purple ones. The clean soil which had been washed away was now replaced by heavily infected material, and in addition, on July 23rd, three warts were placed above the largest white tuber. The whole was kept moist. On August 28th the largest white tuber had two small warts on it. The foster stolon, together with the stolon grafted on it, was cut off close to the stalk, the tubers freed from soil and the whole placed in a glass dish and kept for about a fortnight covered with a piece of damp felt to encourage wart formation. At the end of this time each white tuber had at least one small wart on it, but the purple ones (Arran Victory) were quite free from infection. At this point the tubers were preserved in formaldehyde, as the stolon was beginning to rot. A photograph of the specimen is reproduced in Plate XIII, fig. 7.

During the 1925 season four more grafts of this type were made. In each experiment of this type and of type 5, to be described shortly, the plants were grown in soil free from wart disease until tubers at least as large as hen's eggs had been produced on the grafted-on part, then all the tubers, whether arising directly on the foster plant or on the grafted-on part, were surrounded by heavily contaminated material and treated in such a way as to promote wart formation as far as possible.

No. 901. *A King Edward (S) stolon grafted on an Abundance (I) plant.*

The grafted-on stolon produced one tuber weighing approximately 100 gm. Of the 7 eyes 1 was warted.

No. 905. *A King Edward (S) stolon grafted on a Great Scot (I) plant.*

The grafted-on stolon produced:

Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	88	7	7
2	83	6	3
3	47	2	3
Totals	218	15	13

Four Great Scot tubers produced on the immune foster plant were free from wart.

No. 913. *Up-to-Date (S) stolons grafted on a Kerr's Pink (I) plant.*

*Stolon I*

Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	125	0	7
2	112	0	14
Totals	237	0	21

*Stolon II*

Tuber	Weight (gm.)	Eyes warted	Eyes clean
3	53	0	10
4	43	0	6
Totals	96	0	16

One of the "scion" tubers and one of those produced by the foster plant were tested by the method of infection, by summer sporangia, to which reference has already been made(10). The Kerr's Pink tuber remained clean, but the scion tuber became thoroughly infected, showing that the lack of infection on the growing plant was most probably due to the conditions under which the plant was grown not being sufficiently suitable for the production of warts, and not to any effect of the foster plant on the grafted tuber.

The following grafts of type  $5\left(\frac{S}{S/I}\right)$  were made during the 1925 season.



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No. 912. *An Abundance (I) stolon grafted on a King Edward (S) plant.*

<i>Produce of grafted-on stolon</i>			
Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	36	0	6
2	20	0	4
3	8	0	2
Totals	64	0	12

Two of these tubers were tested by Glynne's method of infection by summer sporangia (10) and found immune.

<i>Produce of host plant</i>			
Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	52	3	5
2	43	2	7
3	23	0	8
4	20	0	6
5	10	0	7
Totals	148	5	33

No. 914. *A Kerr's Pink (I) stolon grafted on an Up-to-Date (S) plant.*

The grafted-on stolon produced one tuber weighing 36 gm., all 7 eyes of which were clean, though a tuber borne by a stolon arising on the foster plant, and placed immediately below the other had 3 large eyes infected.

### CONCLUSIONS.

It may be concluded from the above results, showing as they do that the reaction of any piece of tissue towards wart disease is entirely independent of the influence of any foreign tissue grafted upon it, that the cause of immunity from, or susceptibility to, wart disease is innate in the cell and is unable to move thence except by cell division. Further it is not influenced by substances which are able to pass unchanged across a graft fusion layer and permeate the tissues of the other component of the composite grafted plant.

It is thus evident that susceptibility to wart disease is different in nature from susceptibility to crown gall, for Wormald and Grubb (19) have shown that a scion, susceptible to crown gall, may bestow susceptibility on a resistant stock on which it is grafted.

More definite deductions cannot be drawn until our knowledge of the physiological, and particularly the chemical, effects of stock on scion and scion on stock is increased. By considering the above facts,

however, in relation to those which are known concerning the effects of stock on scion and *vice versa*, guidance may be obtained in choosing which lines of attack on the problem are to be avoided as being unlikely to be fruitful, and which are most likely to yield useful results.

The idea expressed by Köhler<sup>(13)</sup> that immunity from wart disease is caused by a compound built up in the leaves and transported thence to all parts of the plant, for instance, is almost certainly wrong.

The fact that at least two alkaloids (and also, according to Daniel, solanine<sup>(7)</sup>) are known to pass across a graft fusion layer, renders unlikely the idea, which has been put forward<sup>(12)</sup>, that solanine is the cause of immunity from wart disease; it seems likely also that the cause of immunity is not explicable in terms of any other compound similar to alkaloids. The same may be said in regard to essential oils and probably tanins (cf. p. 182).

It has already been stated (p. 182) that Barker and Grove have shown that the acidity of apple juice varies according to the stock on which the apple is grafted. It is unlikely therefore that acidity plays any part in rendering varieties immune - a conclusion already reached by Weiss and Harvey<sup>(16)</sup> as a result of hydrion determinations on potato juice, and by Köhler<sup>(12)</sup> as a result of allowing potato tissue to absorb an innocuous indicator and thereby determining hydrion concentration of the cell contents.

We have seen that grafting a small piece of stolon on a plant of opposite reaction towards wart disease caused no change in the reaction of this stolon. In experiments Nos. 528, 901, 905, 913, 912 and 914 the stolon, in swelling into one or more tubers, increased in volume at least a hundredfold. This increase in volume may be looked upon as the final result of a series of synthetic processes following on absorption of inorganic salts by the roots, and of carbon dioxide by the leaves, resulting finally in the building up of the more fundamental constituents of the cells. Hence it is only in this last stage that the building up of immune tissue differs from that of susceptible tissue. In seeking the cause of immunity therefore we must, in all probability, look to one or other of those chemical compounds which constitute the more permanent "structures" of the cell. Since the compound carrying the cause of immunity does not appear ever to leave the cell, and its increase is inseparably connected with cell division, it seems likely to be closely associated with those compounds which are the seats of specificity in general; in other words, immunity from wart disease is a property of protoplasm.

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About the chemistry of protoplasm little is known, but proteins are invariably constituents of it. The extraordinary specificity of these compounds, as revealed by immuno-chemical, and purely chemical, methods, does not seem to be shared by any other class of compound. What is already known of their chemical structures in no way precludes the possibility of their being the seats of specificity in general. A detailed investigation of the proteins of immune and susceptible varieties of potatoes by immuno-chemical methods seems, therefore, to be the next step in the present problem.

### SUMMARY.

It is known that grafting together portions of two different plants is sometimes followed by changes in their chemical contents owing to permeation of each by substances formed in the other.

The present investigation is an attempt to determine by grafting together pieces of immune and susceptible plants whether the cause of immunity from wart disease of potatoes is carried by chemical compounds which can traverse unchanged a graft fusion layer or by those which are unable so to do.

For this purpose all the eight possible types of plants have been built up by grafting together root, shoot and tuber systems from either immune or susceptible plants.

In none of the experiments was the reaction of the tubers towards wart disease seen to be changed.

The deduction is therefore drawn that the cause of the immunity is not carried by any compound which is able to traverse the plant.

In this way the problem is considerably narrowed down, for it is now possible to eliminate many lines of attack as unlikely to be fruitful.

The suggestion is put forward that the examination of the proteins from immune and susceptible varieties by immuno-chemical methods is the most hopeful future line of attack. /

## REFERENCES.

- (1) BARKER, B. T. P. and GROVE, O. (1914). The Composition of Vintage Apple and Pear Juices. 1914-15. *Univ. Bristol Ann. Rept. Agr. Hort. Res. Stn.* 1914, pp. 41-53.
- (2) BROWN, J. W. (1926). Chemical Studies in the Physiology of Apples. V. Methods of Ash Analysis, and the Effect of Environment on the Mineral Constitution of the Apple. *Ann. Bot.* XL, 129-47.
- (3) COLIN, H. et RIOLLE, Y. T. (1918). La greffe Soleil-Topinambour. *C. R. Acad. Sci. Paris*, CLXVI, 856-8.
- (4) COLIN, H. (1921). La greffe Soleil-Topinambour. *Ibid.* CLXXIII, 852-4.
- (5) — (1922). La migration des principes chez les plantes greffées. La greffe Soleil annuel-Topinambour. *Bull. off. dir. Rech. Sci. et Indust. et Invent.* XXXI, 313-18.
- (6) COLIN, H. et FRANQUET, R. (1924). La migration de l'inuline dans les greffes de Composées. *C. R. Acad. Sci. Paris*, CLXXVIII, 518-20.
- (7) DANIEL, L. (1921). Le Greffage, etc. *Bull. off. dir. Rech. Sci. et Indust. et Invent.* No. 20 (Juin, 1921), pp. 354-71.
- (8) DANIEL, L. et RIFERT, J. (1923). Recherches sur les variations du chimisme chez les plantes greffées. *C. R. Acad. Sci. Paris*, CLXXVII, 894-5.
- (9) GRAFE, V. und LANSBAUER, K. (1906). Über die wechselseitige Beeinflussung von *Nicotiana Tabacum* und *N. affinis* bei der Pfropfung. *Ber. d. deuts. bot. Ges.* XXIV, 366-71.
- (10) GLYNNE, M. D. (1925). Infection Experiments with Wart Disease of Potatoes, *Synchytrium endobioticum* (Schilb.) Perc. *Ann. App. Biol.* XII, 34-60.
- (11) JAVILLIER, M. (1910). Sur la migration des alcaloïdes dans les greffes de Solanées sur Solanées. *Ann. l'Inst. Past.* XXIV, 569-76.
- (12) KOHLER, E. (1923). Ueber den derzeitigen Stand der Erforschung des Kartoffelkrebses. *Arb. biol. Reichsanst. für Land- und Forstwirtschaft.* XI, 289-315.
- (13) — (1924). Über die Beziehungen des Kartoffelkrebserregers (*Synchytrium endobioticum* (Schilb.) Perc.) zu seiner Wirtspflanze. *Cent. Bakt.* Ab. 2, LXI, 32-7.
- (14) — (1925). Untersuchungen über den Kartoffelkrebs. *Arb. biol. Reichsanst. für Land- und Forstwirtschaft.* XIII, 385-411.
- (15) LAURENT, C. H. (1906). Sur la variation de la quantité d'atropine et la recherche de cet alcaloïde dans des greffes de Belladone et de Tomato. *Rev. Brit. de Bot.* II, 71-6.
- (16) — (1908). La perméabilité du bourrelet dans les plantes greffées. *Ibid.* (1908), 24-8.
- (17) ROACH, W. A. (1923). Studies in the Varietal Immunity of Potatoes to Wart Disease (*Synchytrium endobioticum* (Schilb.) Perc.). Part I. The Influence of the Foliage on the Tuber as shown by Grafting. *Ann. App. Biol.* X, 142-6.
- (18) WEISS, F. and HARVEY, R. B. (1921). Catalase, Hydrogen-ion Concentration, and Growth in the Potato Wart Disease. *Journ. Agric. Res.* XXI, 589-92.
- (19) WORMALD, H. and GRUBB, N. H. (1924). The Crown-Gall Disease of Nursery Stocks. I. Field Observations on Apple Stocks. *Ann. App. Biol.* XI, 278-91.

## EXPLANATION OF PLATES XII AND XIII.

### PLATE XII.

Fig. 1. Exp. No. 613 (1) (bottom left-hand corner, near sixpence). Heavily warted tissue grafted on the stolon of an immune plant. When the graft was made the "wart" was half its final size. The number of wart disease sori doubled after the graft was made (see text, p. 184).

Exp. No. 613 (2) (right centre), photograph taken August 16th.

Fig. 2. Exp. No. 613 (2), photograph taken August 17th.

Fig. 3. Exp. No. 613 (2), photograph taken September 4th.

Fig. 4. Exp. No. 613 (2), photograph taken September 10th.

(Figs. 2, 3 and 4 taken to same scale.)

In Exp. No. 613 (2) a small tuber 0.5 cm. diameter, having on it one single summer sorus, was grafted on the stolon of an immune plant. No photograph was taken when the graft was made. Figs. 2, 3 and 4 show successive stages in the growth of the "wart," though "fed" by an immune plant, which therefore was unable to inhibit the growth of the wart through the organic food supply (see text, p. 185).

### PLATE XIII.

Figs. 5 and 6. Two stages (taken to same scale) of Exp. No. 616, showing growth of wart on a small potato after being grafted on a stolon of an immune plant. No photograph taken when graft was made (see text, p. 185).

Fig. 7. Exp. 528. Graft union at *A*, cut from stalk of plant at *B*.

A piece of white stolon (below *A*) cut from a susceptible plant was grafted at *A* on the purple stolon (above *A*) of an immune plant. The tubers were produced in soil free from wart disease. Finally when the tubers had arrived at practically their final size they were surrounded with contaminated material. All three tubers on the grafted-on stolon became infected (warts may be seen at *C* and *D*), whereas the ones on the foster stolon remained quite free from wart disease (see text, p. 186).

*(Received November 25th, 1926.)*



Fig. 2.



Fig. 3.



Fig. 1.



Fig. 4.





Fig. 5.



Fig. 6.

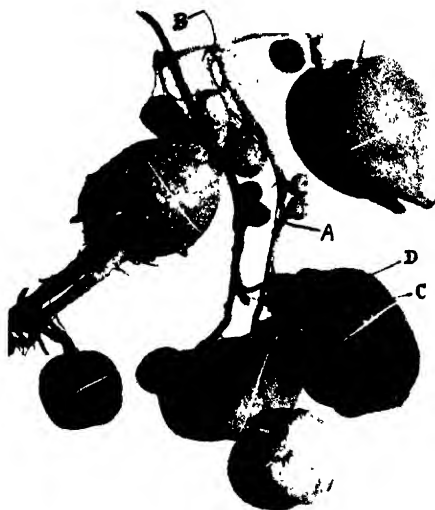


Fig. 7.





## AN OAK LEAF DISEASE CAUSED BY *SCLEROTINIA CANDOLLEANA* (LÉV.) FUECKEL.

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(With Plate XIV.)

DURING the last two years the authors have had under observation a disease which has caused extensive damage on the oak in various parts of Britain, especially in the north. The disease leads to the formation of conspicuous brown blotches on the leaves; in some localities almost the whole of the foliage is discoloured and, in consequence, the impression is given that the tree has been killed. The first specimens were obtained from Perthshire and Aberdeenshire in the summer of 1925 and subsequently in the same year several inquiries regarding the disease were received from widely separated areas in Scotland. In 1926 the disease reached epidemic intensity over the whole of Scotland south of Inverness, and was observed in the greater part of England and also in Wales. Both *Quercus pedunculata* and *Q. sessiliflora* are attacked, and the disease has also been found on nursery plants of *Q. rubra*. It has been recorded by several authors on *Castanea sativa* as well as on *Quercus Robur*.

The disease is caused by *Sclerotinia Candolleana* (Lév.) Fuckel. This species has been known in Britain since 1878<sup>(1)</sup> but has been usually regarded as a saprophyte. Massee in 1910 suggested that living leaves might be infected but it appears that no infection experiments have been previously carried out.

On examination only yellowish hyphae can be seen in the discoloured tissue, during the summer, but after the leaves have fallen, small black sclerotia soon appear and, in consequence, this stage of the fungus was previously known as *Sclerotium Pustula* D.C. and *S. quercinum* Pers.

In the past the fungus has been placed in various genera and the following names are synonymous: *Periza De Candolleana* Lév., *Phialea Candolleana* Quel., *Hymenocypha Candolleana* Phill.

Short accounts of the disease have been previously published in the *Transactions of the Royal Scottish Arboricultural Society* (5) and in the *Gardiner's Chronicle* (6).

The yellow spots on the oak leaves have been first observed towards the end of June. In the early stages they are small and roughly circular (Fig. 1) but gradually increase in size until almost the whole of the leaf may become discoloured. The infected tissue towards the centre of the spot is light brown, and this is surrounded by a darker brown zone. As the season advances the tissue within this dark zone often falls away, leaving a hole in the leaf (Fig. 2). The mycelium in the infected tissue is made up of light yellowish hyphae which ramify in all directions through the cells of the leaf. Secondary fungi are often found on the dead areas, and a species of *Cladosporium* frequently forms a dark dot in the centre of the discoloured tissue. The presence of this fungus was referred to in a previous note (5), where it was suggested that this might possibly represent a conidial stage in the life history of *Sclerotinia Candolleana*. The incorrectness of this suggestion has since been demonstrated by culture and infection experiments.

Sclerotia have not been observed on the leaves while still on the tree, but these soon develop on the fallen leaves (Fig. 2), especially under damp conditions, and they may be also readily obtained by keeping diseased leaves in a moist atmosphere in the laboratory. The sclerotia begin to develop in the leaf tissue and, as they increase in size, burst through the epidermis so that, when mature, they lie freely exposed. A sclerotium usually extends through the thickness of the leaf, but generally develops more strongly towards either the upper or the lower surface. The greater development nearly always takes place towards that side which happens to be uppermost when the leaf is lying on the ground. This ensures that when germination of the sclerotium takes place the fructification is produced freely on the exposed and illuminated surface (Fig. 3). The sclerotia are pulvinate, black, about 0.1 cm. in diameter.

Sclerotia were produced freely in October from a fortnight to three weeks after the leaves were brought into the laboratory. After their formation the leaves with the attached sclerotia were removed from the laboratory and kept outside fully exposed to the weather and, during the winter, were frozen several times. Attempts were made to germinate these sclerotia at various times throughout the winter and spring, but these resulted in failure until the end of May. Germination then took place rapidly, the mature apothecia were produced in about a week

(Fig. 3). Only one apothecium grew out from each sclerotium (Fig. 4). Massee<sup>(3)</sup> describes the development of 3-4 ascophores from the sclerotium but this was never observed. The stalk of the apothecium is slender, yellow in colour, paler towards the base and varies considerably in length, depending on the amount of illumination and the depth of the debris under which the sclerotium is buried. The stalk attains such a length that the apothecium is borne well above the substratum. Diffuse light tends to lengthen the stalk and, when grown under lateral illumination, the growth is always towards the strongest light. The stalks shown in Fig. 3 were up to 4 cm. in length, but these were developed in weak light, while others in full illumination only reached 0.6-1.2 cm.

The apothecia are at first closed but soon open and become flat when mature and are then about 0.4 cm. in diameter. They are reddish to yellowish brown in colour and are usually rather darker on the upper surface. The structure of the hymenium is quite typical of *Sclerotinia* (Fig. 5). The ascospores are elliptical  $7.9 \times 3.4 \mu$ , and the paraphyses are slender and slightly thickened towards the apex (Fig. 6).

An account of the germination of the sclerotia has been given by Rostrup<sup>(1, 2)</sup>. He found that in Denmark germination took place in February and March in the laboratory, but in the forest was delayed until June.

In order to test the parasitism of the fungus, mature apothecia still attached to sclerotia were placed so that the ascospores were naturally shot out upon leaves borne on young plants of *Quercus pedunculata*. As a result brown areas appeared on the leaf in 10-14 days and these on examination were found to contain the hyphae of the fungus.

Cultures on artificial media have been made in the autumn from portions of the diseased leaf, and in the spring from the ascospores. The results in each case are quite similar. A white rather fluffy mycelium is produced; no conidia are formed but sclerotia develop within about a month. These sclerotia are often larger than those found on the leaf and are irregular in form. In plate cultures the sclerotia are not produced in any definite position, but are usually formed at the edge of the medium.

The disease is confined to the leaf and does not spread to the shoots and, in consequence, after the leaves have fallen, the trees are no longer infected. This being the case the collection and burning of the fallen leaves, if thoroughly carried out, should entirely remove the source of infection and hence prevent the disease in the following year.

It appears that wherever the fungus is present the majority of the

leaves become infected and in consequence the disease is very obvious and unsightly. As, however, only leaves are infected, it is improbable that trees will be killed unless the defoliation is very marked and continues over a number of seasons.

Two cases of slight infection in forest nurseries have been observed, on *Quercus pedunculata* and *Q. rubra* respectively. These were both in the vicinity of mature trees which were bearing infected leaves. From a consideration of the life history of the fungus it is obvious that severe infection can only take place within a limited distance of infective material, and it is improbable that the disease will prove to be serious in nurseries, unless these are in proximity to mature trees.

As already pointed out, *Sclerotinia Candolleana* has been recorded fairly frequently in the past, both in England and Scotland, but it does not appear to have occurred in great abundance. We are unable to account for its appearance as an epidemic during the last two years. It is very improbable that there has been any change in the distribution of either the fungus or its host plant, and we can only suggest that some climatic condition has favoured its rapid increase.

#### BIBLIOGRAPHY.

- (1) BERKELEY, M. J. and BROOME, C. E. (1878). Notices of British Fungi, No. 1723. *Ann. Nat. Hist.* 5th ser. I, 29.
- (2) LIND, J. (1913). *Danish Fungi*. Copenhagen.
- (3) MASSEE, G. (1895). *British Fungus Flora*, IV, 281.
- (4) ROSTRUP, E. (1866). Dyrkningsforsøg med Sclerotier. *Botanisk Tidsskrift*, I, 216.
- (5) WILSON, M. and WALDIE, J. S. L. (1925). A Disease of Oak Leaves. *Trans. Roy. Scot. Arbor. Soc.* xxxix, Pt. 2, 206.
- (6) ——— (1926). An Epidemic Disease of the Oak. *The Gardener's Chronicle*, 3rd ser. LXXX, 106.

#### EXPLANATION OF PLATE XIV.

All figures refer to *Sclerotinia Candolleana* (Lév.) Fuckel. on *Quercus pedunculata*.

Fig. 1. Leaf showing brown spots in early stage of the disease.

Fig. 2. Leaf bearing sclerotia.

Fig. 3. Leaf showing sclerotia bearing apothecia. × about 2.

Fig. 4. A single sclerotium bearing an apothecium. × about 3.

Fig. 5. Longitudinal section through apothecium. × about 70.

Fig. 6. Part of longitudinal section through apothecium, showing asci and paraphyses. × about 200.

(Received November 25th, 1926.)



Fig. 1.



Fig. 3



Fig. 2.



Fig. 5



Fig. 4.



Fig. 6



## THE STRAWBERRY DISEASE IN LANARKSHIRE

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DURING the spring of this year the author's attention was directed to the strawberry disease in Lanarkshire. Field observations were made throughout the season and a considerable amount of experimental work has also been carried out. The results are summarised in the following preliminary report.

At the outset the author wishes to point out that the "Lanarkshire Strawberry Disease" is not necessarily the same as the disease in England. "John Ruskin," a variety cultivated over a large acreage, has been most affected.

In the field two aspects of the disease are apparent, namely, the devastation *en masse* of square yards of strawberry beds, and the obvious diseased condition of individual plants. In estimating the probable cause of the disease it is important that both of these aspects should be borne in mind, since it is clear that the organism or organisms causing the disease not only are capable of quickly destroying individual plants but also are capable of spreading rapidly through the strawberry beds.

It is not intended here to enter into full details concerning the symptoms of the disease, but a short reference to the soil, the strawberry plants, and possible parasites will make the general finding clear. The soil in the infected fields is for the most part heavy and close, sometimes almost a clay, and seldom sufficiently free for healthy root development. It is acid, though not to an excessive degree. Further it lacks humus and has not the proper coherence and openness. Where the disease is worst the drainage is bad; the soil is waterlogged in wet weather and practically hard-pan in dry. As a whole the soil cannot be regarded as being in a first-rate condition.

Such soil conditions necessarily tell against the vigorous healthy growth of the strawberry plants. For the most part in the areas where the disease is prevalent, it cannot be said that they are growing under the most favourable conditions. When lifted the roots of many of the plants, though not actually diseased, are in a weak and lax condition,



consequent on the excessive stiffness of the soil, combined with lack of adequate ventilation. In short the field observations all tend to indicate that the state of the plant itself, and not the causative organism of the disease, is of first importance. It would thus appear that the poor root system with its soft and limp roots, and the starved condition of the plant, are due to unfavourable soil conditions plus fungal competition. These general conditions which tell against the plant favour the fungal flora of the soil, and thus the plants are rendered very susceptible to fungal and other diseases.

In general the diseased plants are of squat stature, with discoloured leaves (often with red margins); they fail to form fruit, and there is a decline in the formation of runners, all of which facts suggest starvation conditions. Examination of the root system shows that the roots may be completely or partially rotted. This is true of any kind of root. In the case of very young roots and rootlets the disease may first appear as blemishes or discolorations, which in a more advanced condition resemble the typical "damping-off" effect of seedlings familiar to growers. In stouter roots there is a somewhat similar state as the dying back takes place. Sections through a diseased root show the presence of fungal filaments which completely exhaust the storage materials of the cortical cells. A section taken above the diseased region shows cortical cells of normal appearance, free from hyphae, and filled with starch grains. In plants where the disease was quite far advanced it was found that the penetrating fungal hyphae were confined to the roots, and that the root stock, petioles and leaves (although showing the results of the disease) were free from fungal hyphae. Young blemished roots and rootlets were found to have hyphae associated with them.

Without prejudging the evidence in any way the observer is struck by the absence in a diseased plant of those fine roots and rootlets which play the chief part in the actual absorption of the mineral salts and water from the soil. It is concluded that the destruction wrought on the fine absorbing roots is responsible for the various signs of starvation perceptible in the green aerial part of the plant and for its final decline and death, in fact for all the symptoms usually associated with "Lanarkshire Strawberry Disease."

#### ORGANISMS CAUSING THE DISEASE.

Wounding organisms of various kinds, such as eel worm, cannot at this stage be ruled out altogether as first causes in the attack; the general evidence, however, points to the fact that the ultimate and rapid

decline in the vitality of the strawberry plant is due to the exploitation of the root system (especially of the fine absorbing roots) by fungal hyphae.

Using the usual mycological technique, attempts were made to isolate the fungus or fungi concerned in the decay. For this purpose roots of various kinds were selected, viz. fine roots 2 cm. long, arising from the base of the stem, old roots showing blemishes or badly attacked, and rootlets arising from these. Where possible the fungi were extracted from the junction of decayed and undecayed tissue after superficial sterilisation with solutions of mercuric chloride or formaldehyde. Eighteen separate extractions of fungi were made in this manner, and on twelve occasions *Pythium* sp. was the only fungus obtained. On the other six occasions the fungi isolated belonged to the Fungi Imperfecti. The *Pythium* cultures were obtained from several batches of plants and extracted at different times. *Pythium* was found associated with both young and old blemished and decayed roots.

Inoculation tests were then carried out. This was done by allowing young strawberry plants (from runners) to strike in pots of sterilised soil, care being taken to avoid contamination from the various external sources. When the plants were well rooted the pots were inoculated with the cultures of *Pythium* which had been extracted. After fourteen to twenty-one days the plants became diseased and typical symptoms were developed. It was found that the *Pythium* was capable of attacking both young and old roots, causing a die-back of the thick roots, and denuding them of their fine fibrous rootlets. Under the same conditions non-inoculated control pots remained healthy. The affected roots in the inoculated pots were taken into the laboratory and the *Pythium* was again isolated from them.

This evidence demonstrates that the *Pythium* is capable of producing the strawberry disease as it occurs in field conditions. These observations however do not necessarily rule out other causes of disease. Some inoculation experiments were also carried out with another of the isolated fungi, and this also proved capable of attacking the strawberry roots. Under conditions unfavourable to the plant it is probable that a number of the soil fungi will prove capable of attacking the roots. It is also probable that under special conditions a great many of the soil saprophytes may develop parasitic tendencies. This tendency will be furthered by any reduced vigour of the crop plants. Further experiments along these lines are in progress. A full account of the isolation of the fungi and methods of inoculation will be set forth in a later publication. In

the field it has been found that there are two periods of fungal attack, namely, spring and autumn. The isolations referred to above were all made from the spring infection. It is possible that isolations from the autumn infection will reveal some additional parasites.

Without stressing such evidence as there is to hand at the present time it appears to the author that whether *Pythium* is or is not the primary agent causing the disease, there can be no doubt that it has the power of exploiting the root system on which the general health of the whole plant depends. Further, it is to be noted that *Pythium* sp. is a fungus which exploits quickly and spreads very rapidly, both of which features are in accordance with the striking aspects of the disease as seen in the field.

In the light of these observations it is clear that the problem is probably an agricultural rather than a mycological one. The main factor involved appears to be the general health of the plant. The fungal attack is to be regarded as an important accessory to the main factor. It is, however, a factor which has become increasingly aggressive owing to the accentuation of certain soil conditions. If the roots reach a sufficient state of laxness and ill-health they are liable to be attacked by any of the parasitic soil fungi. Thus the disease may be due to fungal agency without necessarily being referable to one parasite only in all cases.

In view of this finding, experimental work with the soil is being carried out side by side with the mycological studies.

#### SUMMARY.

1. In the field two aspects of the "Lanarkshire Strawberry Disease" are apparent, namely, the devastation *en masse* of the strawberry beds, and the diseased condition of individual plants.

2. Soil conditions as a whole are unfavourable, and this tells against the development of an extensive and healthy root system.

3. The roots are weak and lax, consequent on the excessive stiffness of the soil. This renders them liable to fungal attack.

4. Roots of all kinds were found to be penetrated by fungal hyphae, and to have hyphae associated with them.

5. Eighteen separate extractions of fungi were made, and on twelve occasions a species of *Pythium* was the only fungus obtained.

6. When inoculated into pots in which strawberry plants had been grown under sterile conditions, the *Pythium* attacked young and old roots, and produced all the symptoms of the disease.

7. The *Pythium* was isolated again in the laboratory from inoculated plants.

8. This evidence demonstrates that the *Pythium* is capable of producing the strawberry disease as it occurs in field conditions. It is concluded that *Pythium* is an important factor in exploiting the root system on which the general health of the plant depends.

9. The problem appears to be an agricultural rather than a mycological one. The main factor involved appears to be the general health of the plant. The fungal attack is to be regarded as an important accessory to the main factor. It is, however, a factor which has become increasingly aggressive owing to the accentuation of certain soil conditions.

10. Experimental work on the soil is being carried out side by side with the mycological studies.

The author's thanks are due to Prof. J. M. F. Drummond for much helpful criticism and advice during the progress of these researches.

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# ANTAGONISM OF MICRO-ORGANISMS AS THE CONTROLLING FACTOR IN THE INHIBITION OF SCAB BY GREEN-MANURING

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(With Plates XV-XVII.)

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## INTRODUCTION.

MILLARD(4) has shown that common scab of potatoes may be inhibited by thoroughly mixing a quantity of green organic matter with the soil before planting. To account for this he put forward the hypothesis that since *Actinomyces scabies* can live saprophytically it will continue to do so as long as there remains a sufficiency of suitable organic residues in the soil, and that in this way a protective action is exerted by the green manure on the potato tubers. There is here a tacit assumption that the fungus behaves in a similar manner in nature as in artificial media where its habit is to form a compact lichenoid-like growth on the substratum rather than to spread in the manner of most fungi. For some time it has been felt that some other reason might be found to underlie the phenomenon. It is well known that where, in artificial culture, two micro-organisms are grown on the same plate culture or on the same slope, the one may become dominant even to the total extinction of the

other. The dominant organism is naturally that which is the better adapted for growth on the media employed. A like competition takes place among micro-organisms in the soil, and it occurred to us that therein might be found a solution of the problem. "Scabbing soils" are naturally low in organic content, and, as an example, we may mention one of the worst "scabbing soils" with which we are acquainted where the loss on ignition was only 3.8 per cent. Organic matter whether of animal or vegetable origin quickly disappears in such soils and its decomposition is accompanied by great increases in the micro-flora. Conn(1) cites an increase in *Actinomyces* content of 10,000,000 per gram when grass roots were added to the soil. Millard and Whittle in some unpublished work have found that the initial increase of the *Actinomyces* after green manuring is extremely rapid in light soils. Naturally those species which will most readily respond to this growth impetus will be the obligate saprophytes. There seems little doubt, however, that parasitic species are also able to live on organic remains in the soil, since, as shown by Jones and Edson(2), B. F. Lutman(3), and Millard and Burr(6), scab may appear on potato crops grown in soils which have either never grown a root crop or have been out of cultivation for 50 years or more. Moreover, Millard and Burr(7) have recently shown that there is a wide variation in the degree of parasitism shown by certain members of the group, and it may well be that the saprophytic capacity of any species is in inverse ratio to its parasitic potentiality.

Thus, the addition of green manure to a light soil may be expected to lead, at first, to an increased growth of both saprophytic and parasitic *Actinomyces* types. Again, where the vegetable matter added is in a sufficient state of fineness and brought into a close admixture with the soil, it is reasonable to suppose that a very large proportion of the *Actinomyces* population will eventually become congregated upon it. Here, then, conditions become comparable to those in a mixed artificial culture. There can be little doubt but that the obligate saprophytes will become dominant, and, that being so, it is very possible that either by their secretion of toxic substances or by reason of their greater success in the competition for the available food supply they will depress or even kill off the more weakly growing parasitic species. Such an hypothesis easily lends itself to experimental proof, and the experiments to be described were designed for that purpose.

## PLAN OF EXPERIMENTS.

Pots of sterile soil were inoculated in varying proportions with cultures of two species of *Actinomyces*—one a virulent parasite and the other an obligate saprophyte. In the first experiment, the inoculations were made in such a way that in the series there was a gradually increasing amount of the saprophyte and a correspondingly decreasing amount of the parasite, whilst, in the second and third experiments, the inoculum of the parasite given to each pot was constant, and that of the saprophyte only was made to vary. Other series of pots were set up in each experiment, in which grass cuttings were mixed with the soil before sterilising, and these were similarly inoculated. Potatoes were then grown in the pots.

During the growth of the plants and immediately after lifting, soil samples were taken from the pots and counts made of the two *Actinomyces* species. Unfortunately, no method has so far been evolved whereby any absolute number representing the *Actinomyces* content of the soil can be obtained, but, by following a conventional technique, the counts may be regarded as giving correct relative values—not as between species and species but as between periodical counts of the same species from the different pots. From them it was possible to ascertain the extent of the competition between the organisms, the influence of the grass in this connection and the correlation, if any, between these effects and the scabbing of the respective crops.

*Experiment 1.*

The soil was a light loam having no lime requirement. Three series (*A*, *B* and *C*), each containing 1 pots, were prepared as follows:

Series *A*. Soil only.

Series *B*. Soil and grass cuttings intimately mixed—the amount of grass used being such as would inhibit scab under ordinary field conditions.

Series *C*. Soil and grass cuttings, the latter in twice the quantity used in *B*.

All the pots, when filled, were sterilised at  $3\frac{1}{2}$  atmospheres pressure for 20 minutes and then placed in a greenhouse which had been thoroughly washed and disinfected. For the inoculations the two species of *Actinomyces*, *A. scabies* (Thaxter) Güssow emend. M. and B. and *A. praecox*, from Millard and Burr's(s) stock cultures were selected. The former of

these is a virulent parasite and the latter an obligate saprophyte. Cultures of both species were made on potato plugs, and the pots were inoculated by shaking the cultures into small holes made in the soil. They were then cut into small pieces with a sterile knife and thoroughly mixed in to a depth of 6 inches. The relative proportions of inoculum given in each series were as follows:

Each series. No. of pot	No. of cultures used	
	<i>A. scabies</i>	<i>A. praecox</i>
1	12	0
2	8*	4
3	4*	8
4	0	12

\* It was known from previous experiments that an inoculum of 6 potato plug cultures of this organism would produce a scabbed crop.

After two weeks each pot was planted with a seed potato (variety Field Marshal), which had been sterilised by soaking in a  $\frac{1}{8}$  per cent. solution of formaldehyde for two hours and subsequently allowed to sprout in a sterile box. Later, each pot was given a teaspoonful of a previously sterilised mixture of artificial manures. The benches and the floor in the greenhouse were washed each week with an antiseptic solution and the house was twice fumigated with tetrachlorethane during the experiment. The plants were watered with sterilised tap-

Table I.

*Exp. 1. Degree of scab on crops.*

Series	No. of pot	Description of crop	Virulence
A	1	21 small potatoes all badly scabbed and showing deep brown fissures which, in some cases, covered the whole of the potato	10
	2	7 potatoes of which 5 were scabby	5
	3	10 potatoes, 2 only showing scabs	3
	4	All tubers clean	0
B	1	19 potatoes, 14 badly scabbed showing fissures as in Series A, Pot 1	9
	2	15 potatoes, 8 showing small scabs	3
	3	10 potatoes, 1 only showing scab	1
	4	14 potatoes, all clean	0
C	1	11 potatoes, unfortunately badly attacked by <i>Rhizoctonia solani</i> *, a few small scabs present	1
	2	7 potatoes, 2 scabby	2
	3	8 potatoes, all clean	0
	4	4 potatoes, all clean	0

\* Tubers severely attacked by this fungus are rarely badly scabbed.



water and in every case produced good crops. One sample of soil was taken from each pot during the growth of the plants and one after the potatoes had been lifted for counts of the *Actinomyces* present.

*Results of Experiment 1.* The crops were lifted 20 weeks after planting, and the amount of scab present in each is shown in Table I. The virulence of the disease is indicated by numbers with a maximum of 10.

It is unfortunately not possible to reproduce photographs of all the crops in the different series. Those from Series *B* have been selected and are shown in Fig. 1, where each row of three tubers is representative of the whole crop. The crops from Series *A* were so similar that each might be taken to be a duplicate of that from the corresponding pot in Series *B*. In Series *C*, however, the crops from Pots 2, 3 and 4 were cleaner than the corresponding crops from Series *A* and *B*. The following points appear from the experiment:

(1) That the green manuring in Pot 1, of Series *B*, has not appreciably reduced scab.

(2) That a reduction in scab has taken place in each pot whether of Series *A*, *B* or *C* where inoculations of the saprophytic species have been made. This decrease of scab is so great that in Pot 3 of each series the tubers were practically clean in spite of the inoculations of the parasitic species. In regard to Series *B* and *C*, where green manure had been applied, this result was to be expected, but it was at first very surprising to find that scab had also been inhibited in Series *A* where soil only was used. We attribute this, however, to the fact that a heat sterilised soil is in itself a medium in which rapid Actinomycetal growth will occur and the conditions are thus comparable to that in which an additional food supply has been added.

*Soil Counts.* The two species of *Actinomyces* used in the inoculations behave very differently in culture and no difficulty was apprehended in distinguishing between them on nutrient media. On nutrient potato agar *A. scabies* produces an umbonate fleshy colony which forms no aerial mycelium but stains the medium a deep brown colour. *A. praecox*, on the other hand, produces a white aerial mycelium and does not stain the medium. In practice, however, great difficulty was at first experienced. It should be remembered that during the period of growth of the potatoes, a re-population of the soil with bacteria and fungi was bound to occur. When soil samples are plated out, the bacterial colonies are the first to appear and these may be so numerous as to prevent the subsequent growth of the *Actinomyces*. Even in the plates of the higher dilutions, bacterial colonies exert sufficient influence to change the char-

acters of the *Actinomyces* colonies. Thus, on nutrient potato agar it was found that colonies of *A. scabies* frequently failed to produce any stain, whilst colonies of *A. praecox* sometimes formed no aerial mycelium. Such colonies could therefore only be identified by isolating and growing them in pure culture—a long and tedious process. Again, it was difficult to distinguish between the species when the colonies grew below the surface of the medium. Thornton's synthetic agar and soil extract agar were substituted for nutrient potato agar but were not found to give any more satisfactory results and the counts made were ultimately discarded as valueless. Subsequently, egg albumen agar was found to be a very suitable medium and was employed with success in the later experiments. Here, however, for reasons given later, no appreciable difficulty was caused by bacterial contamination and even nutrient potato agar could be used without trouble.

### Experiment 2.

The procedure followed in this experiment differed from that of Exp. 1 in the following particulars:

(1) The soil was of an extremely light and porous nature in which *Actinomyces* were known to thrive particularly well.

(2) Two series, each consisting of 10 pots (in 5 duplicate pairs) were prepared, Series *A*, of soil only, and Series *B*, of soil mixed with grass cuttings. As this experiment was started in the early spring it was impossible at the time to obtain lawn cuttings, and it did not occur to us that under greenhouse conditions Timothy grass grown in the pots and forked in would have answered the same purpose. However, the inoculations were made in both series of the sterilised soil, and the grass cuttings which became available on March 1st, were then added (after sterilisation) to the pots in Series *B*.

(3) In the inoculations each pot, excepting Nos. 9 and 10, was given

Each series. No. of pot	No. of cultures used as inoculum		
	<i>A. scabies</i>	<i>A. praecox</i>	
1	24	0	} duplicate pots
2	24	0	
3	24	6	} " "
4	24	6	
5	24	12	} " "
6	24	12	
7	24	24	} " "
8	24	24	
9	0	24	} " "
10	0	24	

the same amount of inoculum of the parasitic species, whilst the inoculum of the saprophytic species was increased in geometrical progression in each series. The number of cultures given to each pot is shown in the table on p. 207.

Two additional pots which received no inoculum of either species were included to test the sterility of the soil. It will be noticed that the inoculum of 24 plug cultures of the parasitic species was an extremely heavy one and constituted a very severe test for the theory, even where the saprophyte was added in correspondingly heavy amounts.

(4) Two varieties of potatoes, Epicure and Sharp's Express, were used and one of each was planted in the duplicate pots on March 5th.

In all other respects the technique of the experiment was similar to that of Exp. 1. One soil sample was taken from each pot on April 11th, 5 weeks after inoculation of the soil, and another immediately after the potatoes were lifted on August 11th.

*Results of Experiment 2.* These are best appreciated by reference to Fig. 2 where again each row of tubers is representative of the respective crop or crops from duplicate pots of Series *B*. Photographs of four crops only are given since these sufficiently represent the gradation in degree of scab found in the series. The following observations may be made:

(1) In Pots 1 and 2 of each series every tuber was literally a mass of scab (Fig. 2, row 1). As in Exp. 1, green manuring in the absence of a saprophytic *Actinomyces* species has not reduced the degree of scab in the slightest degree.

(2) A gradual reduction in the amount of scab occurred along each series but, in contradistinction to Exp. 1, this was a little more marked in Series *B* (green manured) than in Series *A* (soil only).

(3) In Pots 7 and 8 (Fig. 2, row 3), where the maximum inoculum of the saprophyte was given, scab has not been entirely inhibited. The reduction from that of Pots 1 and 2 was, however, very great and quite comparable to what one might expect under natural conditions in a green manuring experiment on a soil so heavily loaded with the parasite.

*Soil Counts.* The first counts were made on April 11th, 1926. Possibly on account of the enormous *Actinomyces* flora introduced into the pots, the bacterial flora was found to be greatly reduced and bacterial colonies on the plates caused practically no trouble. Both egg albumen agar and nutrient potato agar were therefore used for the platings. On the former medium *A. scabies* produces a large spreading colony, yellow on the underside, and forming a central tuft of white aerial mycelium, whilst *A. praecox* produces a thin flesh-coloured colony on which aerial

mycelium quickly appears, rimming the colony and then forming one or more concentric circles within the first. Where any doubt occurred as to the identity of any colonies, *e.g.* submerged colonies, these were picked off from the millionth dilution plates and grown on nutrient potato agar slopes. Each soil sample was plated in quadruplicate and, excepting in the cases of Pots 2 and 5, where the plates were spoiled by "spreaders," the four series of plates agreed very closely. A fair correspondence was also found between the duplicate pots, *i.e.* Nos. 1 and 2, 3 and 4, and so on, excepting in Pots 7 and 8, Series *A*, and Pots 3 and 4, Series *B*. The figures given in Table II are the averages of the counts from the quadruplicate platings.

Table II.

*Exp. 2. Count of Actinomyces from soil samples taken April 11th, 1926.*

No. of pot. Series <i>A</i>	Average No. of <i>Actinomyces</i> colonies per gram of air-dried soil counted from the millionth dilution plates after 20 days at 24° C.	
	<i>A. scabies</i> (millions)	<i>A. praecox</i> (millions)
1	1.9	0
2	Plates spoiled	—
3	0	52.0
4	0	98.0
5	Plates spoiled	—
6	0	102.5
7	0	14.0
8	0	174.0
9	0	227.0
10	0	234.0
Series <i>B</i>		
1	8.5	—
2	9.5	—
3	0	12.5
4	0	110.0
5	0	169.0
6	0	183.0
7	0	394.0
8	0	124.0
9	0	122.0
10	0	135.0

From Table II the following observations may be made:

(1) The population of *A. praecox* in all pots inoculated with this species is extraordinarily great but there is no indication that this has been appreciably increased by the addition of grass in Series *B*. In this

connection, however, as already pointed out in Exp. 1, due allowance must be made for the fact that we are dealing with sterilised soil in both series and this serves to mask the effect of the grass in Series *B*.

(2) *A. scabies* has shown an increase in the green manured Pots 1 and 2 of Series *B*, over that of the pots containing soil alone in Pots 1 and 2 of Series *A*. Thus, in the absence of saprophytic species, the addition of grass may even lead to an increase of the scab-producing organism.

(3) The table indicates an entire absence of *A. scabies* from every pot which received inoculation with *A. praecox*. This statement, however, needs qualifying since in the greater number of platings counts were only possible from the millionth dilution plates. The figure of the count is most accurately interpreted as meaning absence of *A. scabies* colonies in the millions degree.

A second count using egg albumen agar was made on August 11th, 1926, immediately after lifting the potatoes but was limited to the pots of Series *B*. The results are given in Table III.

Table III.

*Exp. 2. Count of Actinomyces from soil samples taken August 11th, 1926.*

No. of pot. Series <i>B</i>	Average No. of <i>Actinomyces</i> colonies per gram of air-dried soil counted from the millionth dilution plates after 20 days at 24° C.	
	<i>A. scabies</i> (millions)	<i>A. praecox</i> (millions)
1	34.5	0
2	79.5	0
3	2.5	123.0
4	4.0	128.5
5	9.0	148.0
6	4.5	123.0
7	2.5	130.0
8	3.0	141.5
9	0	172.0
10	0	206.0

Here it is obvious that the parasite, though considerably depressed, is by no means killed off as might have been inferred from the first count. The difference between the two counts in respect of *A. scabies* is probably due to the sampling. Thus, the samples for the first count were taken whilst the potatoes were growing in the pots from small holes 3 to 4 inches deep made towards the edge of the pot. The second samples were taken after the soil had been again thoroughly mixed. It seems probable then that *A. scabies* thrives more readily in the lower

and damper soil layers. The results given in Table III are certainly more in accordance with the degree of scab found on the crops.

It was thought very possible that the inhibitory effect of *A. praecox* on *A. scabies* might not have reached its maximum at the time of lifting the crop and that a second crop grown in the same pots might show yet further evidence of this repression. A further experiment was therefore started.

### *Experiment 3.*

Without any renewal of the soil or further inoculation each pot of Series B was replanted on August 20th with a sterilised tuber of the variety Great Scot. The crops were lifted on November 15th and, although the tubers were rather small, the results were of great interest. As regards scab these were as follows:

Pots 1 and 2. Tubers a mass of scab as in the previous crop.

Pot 3. Scab slight.

Pot 4. Scab very severe.

Pots 5 and 6. Scab slight.

Pots 7 and 8. Tubers almost free from scab.

Pots 9 and 10. Tubers quite clean.

Photographs of the crops from Pots 4, 6, 8 and 10 are shown in Fig. 3. In Pot 3 the skin of the tubers was very rough and hard and it seems as though here infection of the tubers had been modified by some secondary cause. With the exception of this pot the series showed a progressive diminution of scab, which was much more pronounced than that of Exp. 2 and entirely in accord with what had been expected.

Soil samples were taken after lifting the crops from Pots 2, 4, 6 and 8, and later, on account of the apparent anachronism shown by the count of Pot 4, a sample was also taken from its duplicate Pot 3. The counts were made on nutrient potato agar and are given in Table IV.

Table IV.

*Exp. 3. Count of Actinomyces from soil samples taken November 18th, 1926.*

No. of pot	Average No. of <i>Actinomyces</i> colonies per gram of air-dried soil counted from the millionth dilution plates after 20 days at 24° C.	
	<i>A. scabies</i> (millions)	<i>A. praecox</i> (millions)
2	225	0
3	—	119
4	7	202
6	—	142
8	—	180

This count reveals two points of interest. The first is the extraordinary further increase in the *A. scabies* content of Pot 2 as compared with the two previous counts of the same soil in Exp. 2. From the lower temperatures of the greenhouse during the two months previous to lifting we might have expected a fall rather than a rise in the *Actinomyces* content of the soil, since it is well known that the parasitic members of the group are most active at relatively high temperatures. Again, such evidence as is available points to the fact that low temperatures are not conducive to spore production. It seems only possible then to account for this increased content of *A. scabies* by the long-continued growth in the soil of its natural host. Millard and Burr<sup>(9)</sup> have shown that this particular species of *Actinomyces* attacks the roots as well as the tubers of the potato and thus the growth of two successive potato crops will have provided a plentiful and well-distributed supply of suitable food to the parasite throughout the year.

The second point of interest is the absence of the parasitic species from the millionth dilution plates of samples from Pots 3, 6 and 8. This result confirms the previous counts and fully establishes the depressing effect of the saprophyte on the parasitic species. Pot 4 furnishes an exception which can only be accounted for by the fact that (along with Pot 3) it received the smallest inoculum of the saprophyte. For some reason too, the soil here was very much damper when the crops were lifted than that of the other pots, and this condition may have favoured the parasitic species. The close correspondence between this count and the degree of scab produced in the pots is well illustrated by the photographs (Fig. 3).

#### DISCUSSION.

It has been shown that in a soil previously sterilised a saprophytic species of *Actinomyces* will depress the growth of a virulent scab producing species to the point at which its parasitic attack is negligible. Whilst, under the conditions of the experiments this inhibitory effect has shown itself in sterilised soil nearly as strongly as in a mixture of sterilised soil and grass, reasons have been advanced for this and there is little doubt that in the field the effect would be very dependent on green manuring. We may now ask wherein lies the incompatibility between these two organisms. The question of soil reaction first suggests itself. Millard<sup>(5)</sup> in his work on the control of scab, found that the addition of green vegetable matter to the soil brought about a slight decrease rather than an increase in acidity, and, more recently, Sanford<sup>(10)</sup> records

a similar result. It was thought desirable, however, to ascertain what change in soil reaction, if any, had taken place in our experiments. On the conclusion of Exp. 3, therefore, estimations of the *pH* values of the soil in each of the pots in Series *B* were made, using Gillespie's method, and the results are given below.

*pH values. Estimations made three weeks after lifting the potato crops in Exp. 3.*

No. of pot	<i>pH</i>		
1	7.3	}	duplicate pots
2	7.7		
3	7.0	}	" "
4	6.9		
5	7.1	}	" "
6	7.5		
7	7.0	}	" "
8	7.3		
9	6.8	}	" "
10	6.7		

The figures from the duplicate pots do not show any kind of agreement, and the deduction which can be drawn from them is that in none of the pots has a reaction been produced which would inhibit the growth of either organism. It would seem then that the incompatibility of the two organisms is due to some factor other than that of soil reaction. Since, on artificial media colonies will grow side by side on the same plate, the writers are inclined to think that the inhibitory effect of the one upon the other is not so much a question of incompatibility as of starvation. On artificial media the parasite is extremely slow in growth compared with the saprophyte, and in a soil well supplied with vegetable matter this difference in character may easily be intensified. Under such conditions it seems very possible for the parasite to be temporarily, if not permanently, starved out. Sanford (11) has shown that in artificial culture the growth of his strains of *A. scabies* was inhibited by various strains of bacteria—in some cases on account of the excessive acidity produced, and in one case to some unknown factor. It seems very feasible, indeed highly probable, that bacteria as well as saprophytic *Actinomyces* may inhibit the development of the parasitic *Actinomyces* species. The present experiments, however, do not afford any support to the theory. Thus, in the green manured pots which received inoculations of *A. scabies* alone, no diminution of scab was produced in either of the experiments. It must be remembered, however, first, that the *Actinomyces* inoculations were very heavy, and secondly, that the



bacterial flora of the soil in the pots would only slowly establish itself. Even where bacterial development was stimulated by green manure, the competition with the already-established *Actinomyces* would not be comparable to that which could occur under natural conditions. Obviously, the action of bacteria in this connection is open to the same tests as those herein described, and these would give more reliable results than can be obtained from laboratory cultures.

Although green manuring has certainly established itself in this country as a means for growing clean potatoes—in particular among gardeners and small holders—yet there appear to be a number of cases in which it has not proved effective. Doubtless some of these failures may be attributed to inefficient treatment, but others can scarcely be accounted for in this way. Sanford<sup>(12)</sup>, for example, states that he failed to reduce scab with an application of green rye at the rate of 2½ pounds per potato hill. Such a result would formerly have been very inexplicable to us, but the present work may throw some light upon it. Serious outbreaks of scab rarely occur on acid soils, and generally are most severe on neutral or alkaline soils of a light sandy nature. Where green manure is applied to the latter type of soil the conditions are those which favour a rush to maximum activity of the saprophytic microflora, and a parallel with the heavy inoculations in our own experiments is provided. Sanford gives the *pH* of the soil upon which he experimented as ranging from 4.91 to 5.4, and we suggest that his failure was due to this high initial acid reaction. He records a slight decrease in acidity as a result of green manuring, and shows that in the case of a laboratory experiment with another soil the admixture of rye caused a considerable temporary rise in *pH* value. These observations, however, do not alter the fact that vegetable matter (*e.g.* ploughed-in sod) decomposes very slowly in acid soils, and it is practically certain that under these conditions no inhibition of the scab-producing *Actinomyces* can take place. Lastly, the somewhat remote possibility should not be forgotten that in those soils where green manuring is ineffective the *Actinomyces* species or other organisms which can give a quietus to the scab-producing species may be lacking.

#### SUMMARY.

1. The "preferential food theory" as an explanation of the action of green manure in the control of scab is criticised.
2. Experiments are described in which potatoes are grown in series of pots containing soil only or soil mixed with grass cuttings. In each

series the pots were inoculated with two *Actinomyces* species—the one, *A. scabies* (Thaxter) Güssow emend. M. and B., a virulent scab producer; the other, *A. praecox*, an obligate and prolific saprophyte. In Exp. 1, the inoculum of *A. scabies* was made to decrease and that of *A. praecox* to increase along the series. In Exps. 2 and 3 the inoculum of *A. scabies* was kept constant, and that of *A. praecox* was gradually increased along the series of pots.

3. In the absence of the saprophytic *Actinomyces* species, grass alone exerted no inhibitory action on scab.

4. A reduction of scab was obtained in all pots whether of soil or soil mixed with grass to which an inoculation of *A. praecox* had been given. This reduction was most marked in the case of the maximum inoculation with *A. praecox*.

5. Counts of the two *Actinomyces* species were made from soil samples taken from the pots during and after growth of the potato crops, and from these it was found that the presence of the saprophytic species had exerted a very strong depressing effect upon the parasitic species. This depressing effect was most marked after a whole season's growth as shown by Exp. 3. Here, on the millionth dilution platings from a pot inoculated with *A. scabies* alone, 225 colonies grew but no colonies of this species were found on the plates of the same dilution in the case of three pots to which inoculations of *A. praecox* had also been given.

6. The inhibitory effect of *A. praecox* on *A. scabies* is not due to the setting up of an unfavourable soil reaction but is probably caused by a starving out of the weaker organism in competition for the available food supply of the soil.

7. It is suggested that the beneficial effect of green manuring on scab under field conditions is due to similar competitive action and that in this competition the bacteria as well as the *Actinomyces* may play a part.

8. The cases in which green manuring has apparently failed to inhibit scab have been considered from this fresh point of view.

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## REFERENCES TO LITERATURE CITED.

- (1) CONN, J. (1917). *N. Y. Agric. Exp. Sta. Tech. Bull.* LX, 23.
- (2) JONES and EDSON (1901). *Vermont Agric. Exp. Report*, XIV, 231-5.
- (3) LUTMAN, B. F. (1923). *Phytopathology*, XIII, 241.
- (4) MILLARD, W. A. (1921). *Univ. of Leeds and Yorks Council for Agric. Educ. Rep.* 118, pp. 8-20.
- (5) — (1923). *Ann. App. Biol.* x, No. 1, 79.
- (6) MILLARD and BURR (1926). *Ann. App. Biol.* XIII, No. 4, 587-8.
- (7) — — *Ibid.* 590.
- (8) — — *Ibid.* 628-30 and 633-35.
- (9) — — *Ibid.* 589-90.
- (10) SANFORD, C. B. (1926). *Phytopathology*, XVI, No. 8, 536-8.
- (11) — *Ibid.* 541-5.
- (12) — *Ibid.* 536-41.

## EXPLANATION OF PLATES XV—XVII.

- Fig. 1. Exp. 1. Series B. Pots 1 to 4. Each horizontal row of potatoes is representative of the entire crop from one pot. Row 1 at the top is from Pot 1, and the others follow in order.
- Fig. 2. Exp. 2. Series B. Each horizontal row of potatoes is representative of the entire crop from the duplicate pots. Row 1 at the top is from Pots 1 and 2. Row 2 from Pots 3 and 4. Row 3 from Pots 7 and 8 and row 4 from Pots 9 and 10.
- Fig. 3. Exp. 3. Pots 4, 6, 8 and 10. Each group is representative of the crop from a given pot. The groups are: top, left, from Pot 4; top, right, from Pot 6; bottom, left, from Pot 8; bottom, right, from Pot 10.

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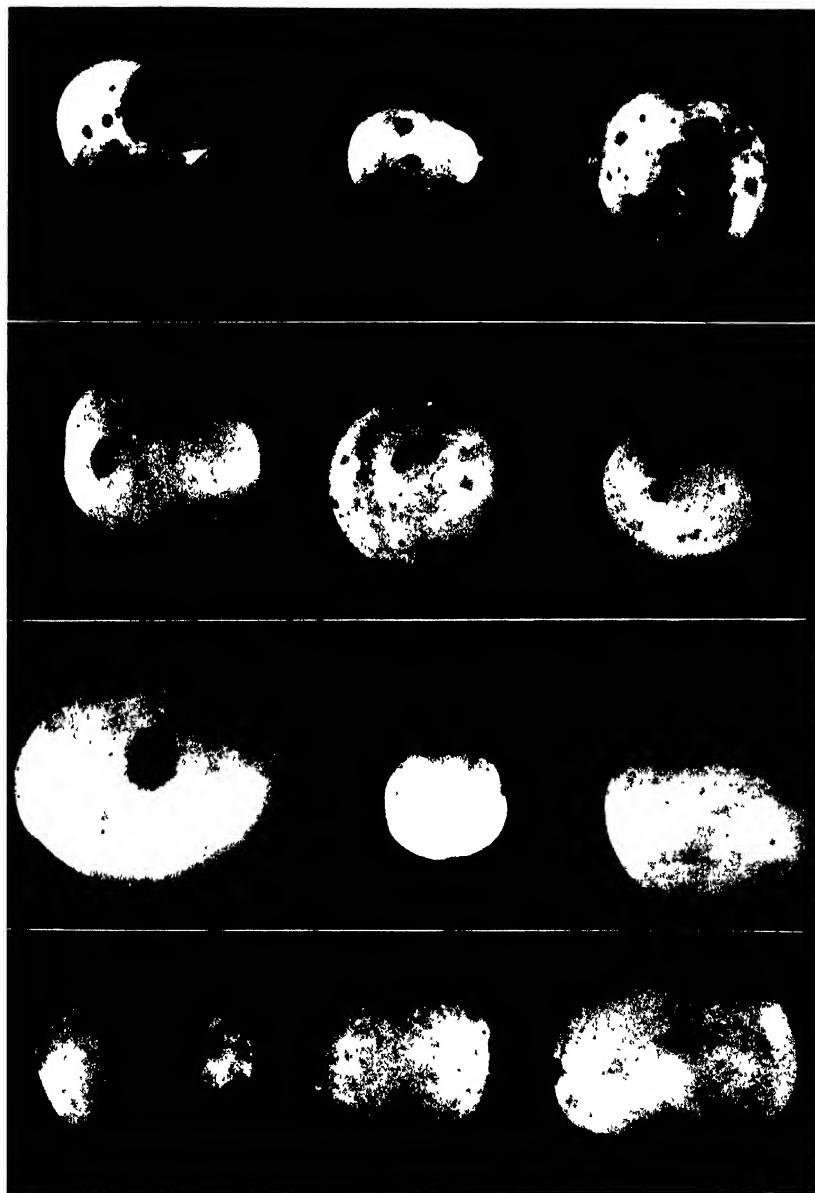


Fig. 1.



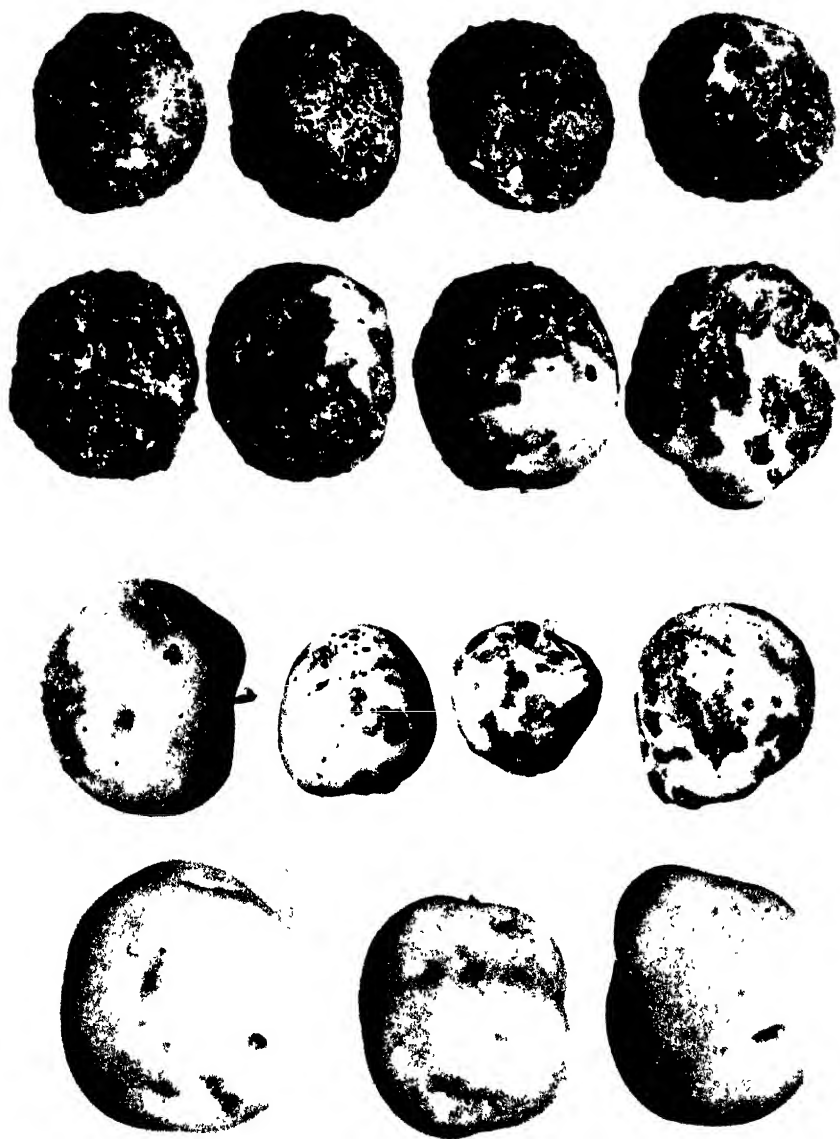


Fig 2.



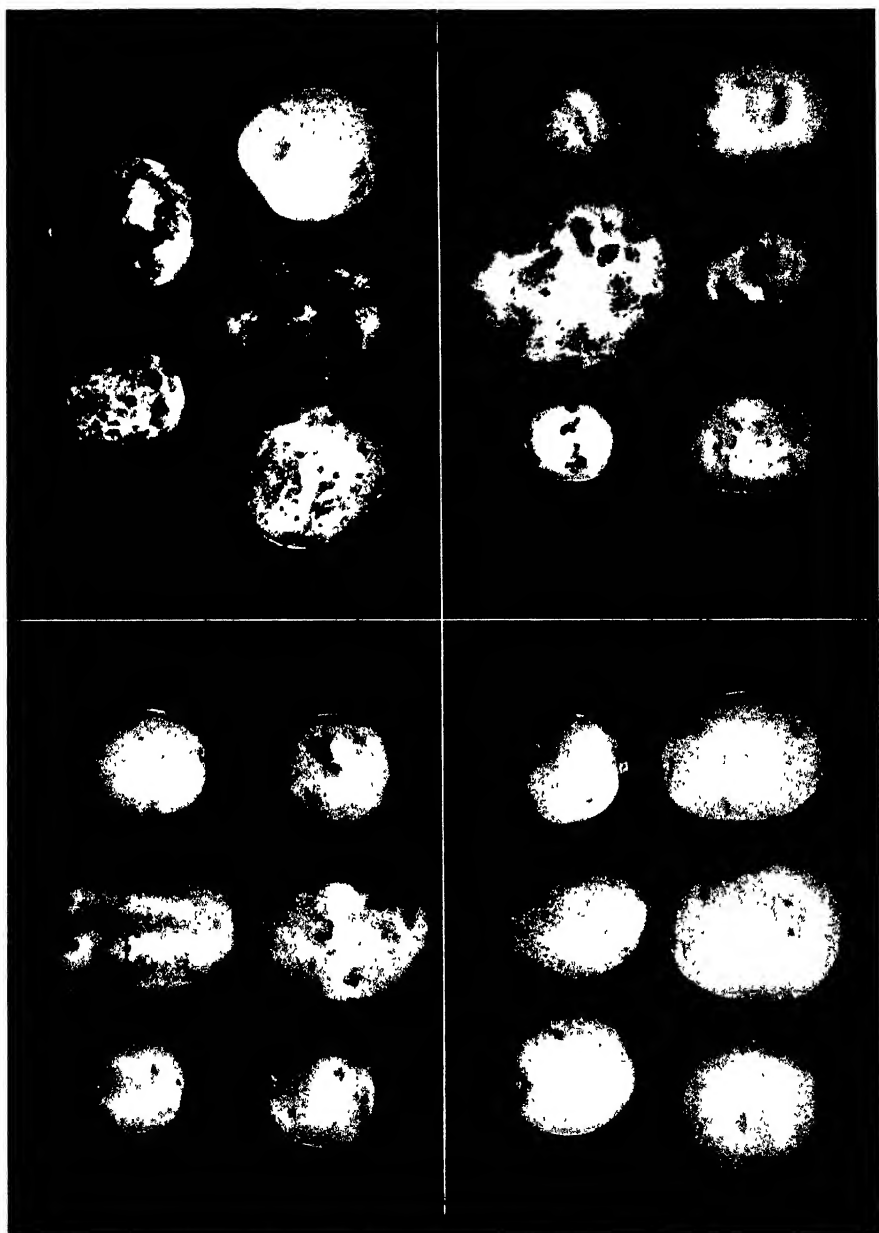


Fig 3.

MILLARD & TAYLOR. —INHIBITION OF SCAB BY GREEN-MANURING (pp. 202—216).





## STUDIES ON CONTACT INSECTICIDES

### PART V. THE TOXICITY OF THE AMINES AND *N*-HETEROCYCLIC COMPOUNDS TO *APHIS RUMICIS* L.

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(With 6 Diagrams.)

AN account was given in Part III<sup>(3)</sup> of this series of papers of the insecticidal properties of the simpler chlor, nitro and hydroxy derivatives of benzene and naphthalene, compounds of a more or less acidic character. The survey has now been extended to the amines and certain of the nitrogen-heterocyclic group of organic compounds, most of which show basic properties in a greater or less degree.

In Part III definite gradations in toxicity were shown to occur amongst the nitro compounds, and some of them, such as dinitro-phenol and dinitro-*o*-cresol, proved to have marked toxic effects evidently depending upon the make-up of the molecule. We have found similar gradations amongst the compounds dealt with in this paper.

#### EXPERIMENTAL.

The method employed for the determination of insecticidal values has been dealt with in previous papers<sup>(1, 2, 3)</sup>, but a brief résumé is perhaps not out of place. *Aphis rumicis* (the Black Bean Aphis) has again been used as our chief test insect, and as the result of considerable experience we are able to rear large numbers of adult wingless females of this species, during the summer months, which show little individual variation in resistance as judged by the results of many duplicate experiments. The spraying apparatus used<sup>(1)</sup> is designed to give percentage mortality figures under strictly controlled and constant conditions with regard to the amount of liquid sprayed, the pressure employed to produce the spray, and the length of time of contact. A large number of tests can be carried out rapidly and, by testing each compound at a number of concentrations, it is possible to draw diagrammatic curves from the

results, plotting percentage mortality against concentration, and thus to obtain a convenient means of comparing toxicities.

In making up the mixtures for spraying, we consistently use a 1 per cent. solution of saponin in water to ensure satisfactory wetting, and to help emulsification. When the substances to be tested are liquids, they are dissolved in or emulsified with the saponin solution. Solids insoluble in water are, whenever possible, dissolved in a small quantity of benzene and then emulsified with the saponin solution.

Control experiments, many times repeated, have shown that 1 per cent. saponin solutions, with or without the addition of benzene in amounts much greater than are used in the liquids sprayed, are without toxicity to *A. rumicis*. It has occasionally been necessary to add small amounts of other solvents; in such cases special control tests are carried out.

After spraying, the insects are placed within reach of fresh bean foliage and are examined on the two (or, if necessary, three) following days. At each examination, counts are made under four headings: "unaffected," "slightly affected," "moribund" and "dead." In the tables, except in the cases of unimportant compounds, the full data for the four categories are given, but in the diagrams, the sums of the figures in the last two categories only are plotted against the concentration. This procedure perhaps involves inadequate grading of comparative toxicities in some cases, but since "slightly affected" insects almost invariably recover and "moribund" insects almost invariably die<sup>1</sup>, we believe that it at all events does not exaggerate, though it may tend to minimise toxicity.

Mr H. M. Morris collaborated with us in the earlier stages of this work and we wish to express our appreciation of his help and to record our thanks.

#### *The Amines.*

*Quaternary compounds.* Both aliphatic and aromatic amines have been tested. In the former of these groups chief attention was given to the tetramethyl and ethyl compounds, whereas amongst the aromatic compounds a general survey covering a large number of derivatives has been made.

The results obtained with the aliphatic and certain aromatic quaternary compounds are set out in Table I; the figures for tetramethyl and tetraethylammonium hydroxides and salts are plotted in Diagram 1.

<sup>1</sup> There is an exception in the case of compounds exhibiting anaesthetic action. These are specially noted.

Table I.

*Toxicities to A. rumicis of certain quaternary ammonium compounds and trimethylamine.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration*		No. of tests	N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.						
Trimethylamine hydrochloride $N(CH_3)_3HCl$	5.0	.084	—	—	—	—	100	100
	2.5	.042	—	—	—	20	80	100
	1.0	.017	—	10	—	40	50	90
	0.75	.013	—	10	—	20	70	90
	0.5	.008	—	30	—	—	70	70
	0.25	.004	—	10	40	30	20	50
Tetramethylam- monium hydroxide $N(CH_3)_4OH$	2.0	.022	1	—	—	—	100	100
	1.0	.011	3	3	7	3	87	90
	0.75	.0083	2	—	5	20	75	95
	0.5	.0055	3	3.5	13	23.5	60	83.5
	0.35	.0038	2	41	12	18	29	47
	0.2	.002	2	45	20	15	20	35
	0.1	.001	3	67	3	20	10	30
	0.05	.0005	3	80	6.5	3.5	10	13.5
Tetramethylam- monium chloride $N(CH_3)_4Cl$	2.0	.012	1	—	—	—	100	100
	1.0	.01	3	—	—	—	100	100
	0.75	.0083	3	—	—	—	100	100
	0.5	.0055	3	7	3	10	80	90
	0.35	.0038	2	10	5	—	85	85
	0.2	.002	2	10	—	35	55	90
	0.1	.001	3	70	10	13	7	20
	0.05	.0005	3	83.5	3	10	3.5	13.5
Tetramethylam- monium sulphate $(N(CH_3)_4)_2SO_4$	1.0	.011	1	—	—	10	90	100
	0.75	.0083	1	—	—	10	90	100
	0.5	.0055	1	—	20	20	60	80
	0.35	.0038	1	—	30	10	60	70
	0.2	.002	1	40	—	10	50	60
	0.1	.001	1	50	—	20	30	50
	0.05	.0005	1	90	—	—	10	10
Tetraethylam- monium hydroxide $N(C_2H_5)_4OH$	2.0	.014	3	23.5	13	10	53.5	63.5
	1.5	.0102	1	40	—	10	50	60
	1.0	.007	3	30	10	13	47	60
	0.75	.0051	3	57	3	3	37	40
	0.5	.0034	3	65.5	3.5	3.5	27.5	31
	0.35	.0024	2	80	—	10	10	20
	0.25	.0017	1	80	—	10	10	20
	0.2	.0014	2	80	—	15	5	20
Tetraethylam- monium chloride $N(C_2H_5)_4Cl$	2.0	.014	2	15	10	15	60	75
	1.5	.0102	1	30	—	—	70	70
	1.0	.0068	2	35	—	35	30	65
	0.75	.0051	2	25	10	15	(55)	(70)
	0.5	.0034	2	60	15	10	15	25

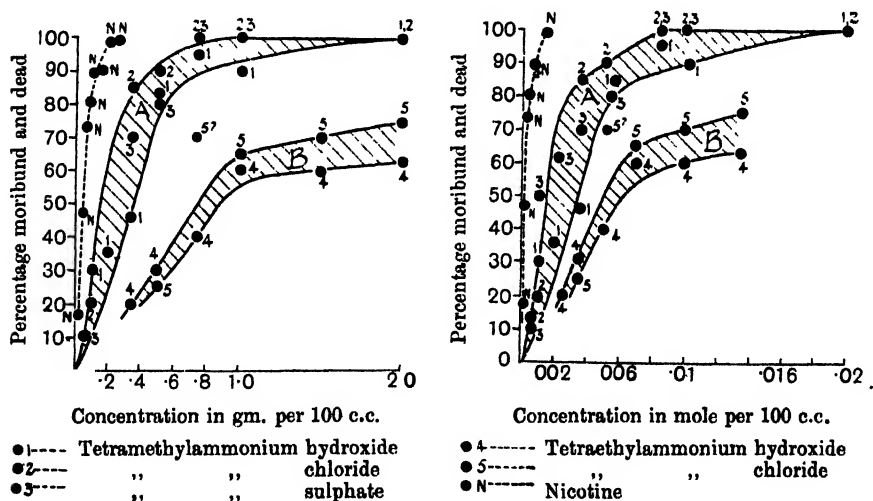
\* The concentrations in all cases are given in terms of the respective bases.

Table I (continued).

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration		No. of tests	N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.						
Trimethylaniline hydroxide $C_6H_5N(CH_3)_3OH$	10.0-2.5	0.065-0.016	3	—	—	—	—	100
	2.0	0.013	1	—	10	—	90	90
	1.0	0.0066	2	16	16	5	63	68
	0.75	0.0049	1	50	20	—	30	30
	0.5	0.0032	2	90	—	5	5	10
Trimethyl- $\alpha$ - naphthylamine hydroxide $C_{10}H_7N(CH_3)_3OH$	2.0	0.01	1	—	—	—	100	100
	1.0	0.005	1	20	10	20	50	70
	0.75	0.0037	1	20	20	—	60	60
	0.5	0.0025	1	40	20	—	40	40
	0.25	0.0012	1	100	—	—	—	—
Trimethyl- $\beta$ - naphthylamine hydroxide $C_{10}H_7N(CH_3)_3OH$	2.76	0.014	1	10	10	10	70	80
	1.38	0.0078	1	10	10	10	70	80
	0.69	0.0039	1	20	30	—	50	50
	0.51	0.0025	1	70	—	—	30	30
	0.34	0.0015	1	100	—	—	—	—
Dimethylbenzyl- phenyl-ammonium hydroxide $N(C_6H_5CH_2)(C_6H_5)(CH_3)_2OH$	2.5	0.011	1	—	—	—	100	100
	1.0	0.0044	1	—	—	—	100	100
	0.5	0.0022	1	78	—	11	11	22

Ammonium hydroxide was not materially toxic at a concentration of 9 gm.  $NH_3$  per 100 c.c. Ammonium chloride, ammonium sulphate, sodium chloride, sodium sulphate had little toxic effect at concentrations of 2 gm. per 100 c.c. (the highest concentration tested).



In both table and diagram, the concentrations are expressed in grams and gram-molecules of the base per 100 c.c. of solution sprayed. The hydroxide, chloride and sulphate of tetramethylammonium and the hydroxide and chloride of tetraethylammonium were tested. Both groups of compounds proved fairly highly toxic to *A. rumicis*. In Diagram 1, the results for the tetramethyl compounds fall within the shaded area *A*, the hydroxide on the right and the chloride on the left margin, the sulphate being intermediate between the two; and those of the tetraethylammonium compounds fall in the shaded area *B*. In both cases the hydroxides appear slightly less toxic than the salts, though these differences are scarcely significant. It is noteworthy that such apparently innocuous derivatives as the chlorides of these bases should have so high a degree of toxicity. Tetraethylammonium was distinctly slower in its action than tetramethylammonium, the former showing little toxic action until after 24 hours.

It has long been known that tetramethylammonium possessed marked physiological effects upon the higher animals. The classical researches of Crum Brown and Fraser<sup>(4)</sup> in 1869 showed it to be a powerful poison with a paralysing action on the peripheral terminations of motor nerves and to have a direct action on the cerebro-spinal system. In more recent years, the researches of Barger and Dale<sup>(5)</sup> on "Chemical Structure and the Sympathomimetic Action of Amines," and of Burn and Dale<sup>(6)</sup> on the "Action of certain Quaternary Ammonium Bases," have shown that tetramethylammonium possesses certain physiological effects similar to those of nicotine, in marked contradistinction to tetraethylammonium. Burn and Dale, summarising their conclusions, say "Tetra-methylammonium hydrate . . . possesses the nicotine-like action in a very intense degree, being approximately as active in its stimulation of ganglion cells as nicotine itself; it also possesses a weaker but well-marked action of the muscarine type. . . . On the ganglion cells, on the other hand, though the tetraethyl compound has no appreciable stimulating action in ordinary dosage, it exhibits the secondary paralytic phase of the nicotine-action almost as powerfully as the tetramethyl compounds."

In insecticidal tests one would expect the paralysing action to be of importance, and if this phase in the physiological action of the two compounds, tetramethyl- and tetraethylammonium, were not strongly differentiated, that there would be only insignificant differences in their respective toxicities. Our results show that, weight for weight and mole for mole, the tetramethyl compound is distinctly more toxic to

*A. rumicis* than the corresponding ethyl compound, indicating that in all probability the whole series of physiological effects, in which tetramethylammonium is similar to nicotine, is of importance in determining its toxicity as a contact insecticide.

Richardson and Smith (7) also tested the chlorides of these bases upon *A. rumicis*, using a different technique, and determined the minimum concentration killing 95 per cent. They found the tetramethylammonium compound to be almost seven times as toxic as the tetraethyl derivative mole for mole.

The relatively high toxicity of the tetramethyl compounds as compared with the corresponding ethyl derivatives is not readily explained, and there is little in the literature which seems helpful. In the theory of Overton (8) and Meyer (9), an attempt is made to correlate narcotic action with the partition coefficients of compounds (*i.e.* the ratio of solubility in lipoid or oil to solubility in water). Some work on the possibility of a relationship between partition coefficients and insecticidal action is discussed in a later paper in connection with another group of compounds—the fatty acids. In the absence of direct experimental work on these lines on the amines, it is impossible to say whether Overton and Meyer's theory would offer any help in accounting for the difference in toxic action between tetramethyl- and tetraethylammonium. The theory has been subjected to much criticism, and, expressed in its simplest form, it seems unlikely to be helpful, for it is difficult to believe that any close correlation exists between the partition coefficients and the insecticidal action of these substances. Harvey (10) has shown that weak bases penetrate tissues of marine organisms more rapidly than strong bases, and that tetraethylammonium hydroxide, a highly dissociated base, cannot penetrate sea-urchin's eggs and is less toxic than mono-ethylamine hydroxide, a slightly dissociated base. The effect of tetramethylammonium hydroxide is not discussed in his paper. According to Harvey, the degree of dissociation can act in two countervailing ways: the higher it is the lower the penetration power, and in consequence the toxic action; but provided penetration does take place, a more highly dissociated base shows a greater toxicity than one of a lower degree of dissociation. In the case of tetramethyl- and tetraethylammonium, however, both bases are highly dissociated.

It is a matter of common knowledge that methyl and ethyl groups, especially in the alcohols, differ in their physiological effects, and the investigations of Flusin (11) on the penetration of the pig's bladder by

certain alcohols showed that, whereas methyl alcohol penetrates readily, this membrane is almost impermeable to ethyl alcohol. Thus, despite the close similarities between the methyl and ethyl groups in both chemical and physical properties, we are forced to the conclusion that both biochemically and biophysically they must react with some element of the living organism in different ways. The difficulty of accounting for the differences in the physiological action of tetramethyl- and tetraethylammonium is summed up by Dale<sup>(12)</sup> in the words "we are driven back on a conception of some peculiar conformation of the reactive surface to which the methyl groups are adjusted which the ethyl groups will not fit," and they apply equally well to the differences shown by the compounds in their insecticidal action.

In Table I the results obtained with trimethylamine hydrochloride are also given; it proved only slightly less toxic than tetramethylammonium chloride, a result which agrees with that of Richardson and Smith (*loc. cit.*). In addition, certain other quaternary compounds, such as phenyl-trimethylammonium hydroxide, and  $\alpha$ -naphthyl-trimethylammonium hydroxide are placed here for purposes of comparison. Although the experiments with these compounds were only of a preliminary nature, they showed that methyl cannot be replaced by the aromatic radical phenyl, or  $\alpha$ -naphthyl, with any advantage, the resulting compounds being on the whole less toxic both weight for weight and mole for mole than tetramethylammonium hydroxide. The compound dimethylbenzylphenylammonium hydroxide is of the same order of toxicity as tetramethylammonium hydroxide when molecular comparisons are made, but it is much less toxic when compared on a gram for gram basis.

Thus, so far as we have been able to test this series, the tetramethylammonium radical is unique in its toxic action.

*The aromatic amines (aniline derivatives).* Table II and Diagram 2 show the results obtained with some aromatic amines.

A large number of the more readily available amines were tested. It would be impossible to graph in any clear way the whole of the results obtained, and the tables have been shortened in many cases, by giving only the outstanding data. In Diagram 2 a shaded area is given within which practically all the aliphatic aniline derivatives fall. The isobutyl and iso-amyl anilines appear on the margin to the left, methyl aniline and toluidine fall to the right of this area, aniline itself being well outside to the right; dimethylaniline practically bisects the area. The labour required to differentiate further between these compounds did not seem



Table II.

*Toxicities to A. rumicis of the aromatic amines.*

[N=not affected; S=slightly affected; M=moribund; D=dead ,

Substance	Concentration		No. of tests	N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.						
Aniline	10.0	.11	1	—	—	—	100	100
$C_6H_5NH_2$	5.0	.053	1	—	10	20	70	90
	4.0	.042	1	30	20	10	40	50
	3.0	.032	1	70	10	10	10	20
	2.0	.021	1	80	10	—	10	10
	1.0	.011	1	90	10	—	—	—
	5.0-2.5	.047-.023	5	—	—	—	—	100
Methylaniline	2.0	.019	1	—	—	70	30	100
$C_6H_5NH(CH_3)$	1.0	.0093	2	50	10	15	25	40
	0.75	.007	1	80	—	20	—	20
	0.5	.0047	2	95	—	5	—	5
	0.25	.0023	2	90	10	—	—	—
	4.0	.032	2	—	—	—	100	100
Dimethylaniline	3.0	.024	2	—	—	15	85	100
$C_6H_5N(CH_3)_2$	2.5	.02	1	—	—	—	100	100
	2.0	.016	2	5	—	32	63	95
	1.0	.008	3	30	—	46.7	23.3	70
	0.5	.004	3	80	—	6.7	13.3	20
	0.25	.002	3	90	—	3.3	6.7	10
	5.0-2.0	.041-.016	4	—	—	—	—	100
	1.0	.0082	1	30	—	50	20	70
Ethylaniline	0.5	.0041	1	67	—	11	22	33
$C_6H_5NH(C_2H_5)$	0.25	.002	1	70	10	20	—	20
	5.0-2.0	.034-.013	4	—	—	—	—	100
	1.0	.0067	1	50	—	10	40	50
	0.5	.0033	1	80	—	—	20	20
Diethylaniline	0.25	.0016	1	89	—	—	11	11
$C_6H_5N(C_2H_5)_2$	1.0	.0072	1	—	—	—	100	100
	0.75	.0054	2	—	—	—	100	100
	0.5	.0036	2	—	—	10	90	100
	0.25	.0018	2	10	—	30	60	90
	0.125	.009	2	65	—	10	25	35
	0.1	.0072	—	—	—	—	—	—
	0.5	.0036	1	70	20	10	—	10
Diphenylamine	1.0	.0059	3	—	—	20	80	100
$(C_6H_5)_2NH$	0.75	.0045	2	5	—	35	60	95
	0.5	.003	2	—	5	35	60	95
	0.35	.0021	1	10	10	10	70	80
	0.25	.0015	2	45	5	35	15	50
	0.2	.0012	—	—	—	—	—	—
	0.1	.00059	2	48	—	38	14	52
Triphenylamine	1.0	.0041	—	Not appreciably toxic				
$(C_6H_5)_3N$	1.0	.0054	1	—	—	—	100	100
	0.75	.004	1	—	—	—	100	100
	0.5	.0027	1	—	—	20	80	100
	0.35	.0019	1	50	—	20	80	100
	0.2	.0011	1	60	—	—	40	40
	0.1	.00054	1	70	—	20	10	30
	0.1	.00054	1	70	—	20	10	30
Methyldiphenylamine	1.0	.0054	1	—	—	—	100	100
$(C_6H_5)_2NCH_3$	0.75	.004	1	—	—	—	100	100
	0.5	.0027	1	—	—	20	80	100
	0.35	.0019	1	50	—	20	80	100
	0.2	.0011	1	60	—	—	40	40
	0.1	.00054	1	70	—	20	10	30

Table II (continued)

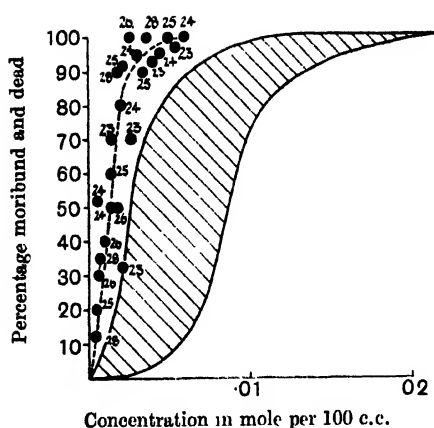
[N=not affected; S=slightly affected; M=moribund; D=dead.]

Substance	Concentration		No. of tests	N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.						
Benzylamine (C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> )NH <sub>2</sub>	2.0.	.019	—	Not appreciably toxic				
Dibenzylamine (C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> ) <sub>2</sub> NH	5.0-1.0	—	6	—	—	—	100	100
	0.5	.0025	1	—	—	—	100	100
	0.25	.00125	1	—	30	40	30	70
Tribenzylamine (C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> ) <sub>3</sub> N	2.0	.007	—	Not appreciably toxic				
Benzylaniline C <sub>6</sub> H <sub>5</sub> \ NH \ C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	1.0	.0054	4	2.5	—	30	67.5	97.5
	0.75	.004	3	7	—	50	43	93
	0.5	.0027	3	13	17	37	33	70
	0.35	.0019	1	30	40	30	—	30
	0.2	.0011	1	50	30	10	10	20
	0.1	.0054	3	57	27	6	10	16
Dibenzylaniline (C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> ) <sub>2</sub> -N \ C <sub>6</sub> H <sub>5</sub>	1.0	.004	—	Not appreciably toxic				
Methylbenzylaniline C <sub>6</sub> H <sub>5</sub> \ NH \ C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -NCH <sub>3</sub>	1.0	.005	1	—	—	10	90	100
	0.75	.0037	1	—	10	10	80	90
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -NCH <sub>3</sub>	0.5	.0025	1	10	—	20	70	90
	0.25	.0012	1	10	30	10	50	60
	0.1	.0005	1	30	50	10	10	20

The following were tested at a number of concentrations and were found to secure 90-100 per cent. of deaths at strengths ranging from 1-2.5 gm. per 100 c.c.; the points representing the toxicities at different concentrations all fall within or very near the shaded area in Diagram 2: *o*- and *p*-toluidine, dimethyl-*o*- and *p*-toluidine, *o*-, *m*- and *p*-xylydine, di-*n*-propylaniline, *iso*- and di-*iso*propylaniline, *isobutyl*aniline, *iso*- and di-*iso*amylaniline, dibenzylaniline, *o*- and *p*-anisidine, *o*- and *p*-phenetidine, monomethylaniline nitrosamine, nitrosomonomethylaniline nitrosamine, *p*-nitrosodimethylaniline.

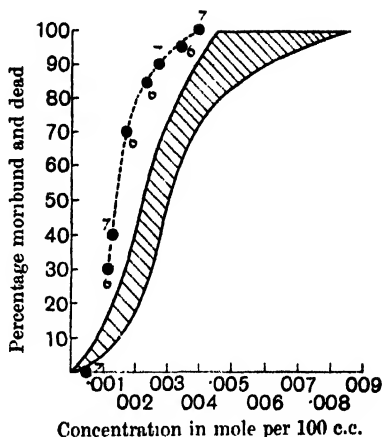
The phenylenediamines (*o*, *m* and *p*) were not toxic at a concentration of 1 per cent. Aminodiphenylamine was not appreciably toxic at 1 per cent. Triphenylamine was much less toxic than diphenylamine, having little effect at or below a concentration of 1 per cent.

The *m*- and *p*-nitranilines, owing to their relatively low solubility in benzene, were difficult to work up into a form suitable for spraying, but were not appreciably toxic at the strengths tested. The following compounds were also tested:  $\beta$ -phenylhydroxylamine, hydrazobenzene, azobenzene, azotoluene, azoxybenzene, azoxytoluene.  $\beta$ -phenylhydroxylamine and azoxybenzene secured 90-100 per cent. of deaths at concentrations ranging from 0.75 to 1.0 gm. per 100 c.c.; this was also the case with diazoaminobenzene which was more toxic than aminoazobenzene.



The other aromatic aniline derivatives fall within the shaded area, with the exception of aniline, which falls outside to the right

Diagram 2. Toxicities of the aromatic amines to *Aphis rumicis*.



$\alpha$ -naphthylamine, methyl-, dimethyl-, and ethyl- $\alpha$ -naphthylanilines fall within the shaded area

Diagram 3. Toxicities of  $\alpha$ -naphthylamine derivatives to *Aphis rumicis*

worth while. The main deduction to be drawn is that in the case of aniline the substitution of methyl, methoxyl and ethoxyl groups in the benzene ring or of aliphatic groups on the nitrogen do not give rise to any great increases in toxicity although all are more toxic than the original compound. On the other hand, aromatic nuclei, substituted on the nitrogen, give rise to marked increases in toxicity over the parent substance; thus diphenylamine, methyldiphenylamine, benzyl-aniline, methylbenzylamine are more toxic than aniline or any of the aliphatic anilines, both weight for weight and mole for mole. The phenyl group appears to be of greater significance than the benzyl group in this respect, but the points on the diagram come so near together that the toxicities of all four of these substances can be fairly well represented by one curve. Triphenylamine proved less toxic than diphenylamine and dibenzyl- and benzalaniline less toxic than benzylaniline.

The results obtained with the benzylamine derivatives are particularly interesting, for they show toxic relationships *inter se* of the same kind as those shown by the phenylamines. Dibenzylamine is considerably more toxic than either benzylamine or tribenzylamine.

This is analogous to the effects obtained with the phenylamines and benzylanilines; *i.e.* if in each of these series, ammonia ( $\text{NH}_3$ ) is taken as the basic radical, the compounds in which two hydrogen atoms are replaced by aromatic nuclei are more toxic than those in which one or three are substituted. We have therefore the following orders<sup>1</sup> of toxicity:

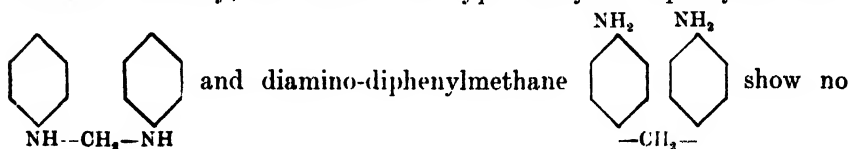
phenylamine(aniline) < diphenylamine > triphenylamine.

phenylamine(aniline) < benzylaniline > dibenzylaniline.

benzylamine < dibenzylamine > tribenzylamine.

These relationships raise the question whether the last hydrogen attached to the nitrogen which remains unsubstituted in these compounds has not a definite significance in determining the toxicity. This hydrogen, however, can be replaced by methyl without loss of toxic properties, methyl-diphenylamine and methyl-dibenzylaniline have insecticidal properties of the same order as diphenylamine.

The substitution of a second amino group in the *o*, *m* or *p* position, or of chlorine or hydroxyl groups, in the aniline ring produces no marked increase in toxicity, and bases of the type methylene-diphenyl-di-imide



show no appreciable toxicity at a concentration of 1.0 per cent. This is also true of benzylethylaniline, formyl-diphenylamine, the benzidine and tolidine bases, anhydro-formaldehyde-aniline and anhydro-formaldehyde-*o*-toluidine.

The introduction of a nitro group in the *o*-position to the amino group in aniline has a pronounced effect on toxicity. *o*-Nitraniline is one of the most toxic derivatives of aniline tested. On a gram-molecular scale the points happen to fall close to the curve showing the toxicity of diphenylamine (see Diagram 4). The *m*- and *p*-nitro derivatives were not sufficiently soluble to permit of testing at any concentration at which they showed toxicity.

The nitroso compounds present few points of interest; the nitrosamine of methylaniline, the nitrosamine of nitroso-methylaniline and *p*-nitroso-dimethylaniline were toxic at concentrations of about 0.75–1.0 per cent.

<sup>1</sup> The nitrophenols and nitrocresols show effects of a similar type: mono-nitro-phenols < 2 : 4-dinitrophenol > 2 : 4 : 6-trinitrophenol (*Ann. App. Biology*, 1925, xii, 218).

*Naphthylamine derivatives.* In Table III and Diagram 3 are set out the results obtained with  $\alpha$ - and  $\beta$ -naphthylamine and a few of their simple derivatives.

Table III.

*Toxicities to A. rumicis of some naphthylamine derivatives.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration		No. of tests	N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.						
$\alpha$ -Naphthylamine	1.0	.007	1	—	—	—	100	100
$C_{10}H_7NH_2$	0.75	.005	1	10	—	—	90	90
No solvent: Fine	0.5	.0035	1	10	—	—	90	90
suspensoid	0.375	.0026	1	30	—	—	70	70
Ditto coarse suspensoid				Not materially toxic at 1 %				
Ditto benzene used as	1.0	.007	2	10	—	—	90	90
solvent*	0.75	.005	2	15	—	5	80	85
	0.5	.0035	3	26	10	6	58	64
	0.35	.0025	1	50	—	20	30	50
	0.25	.0018	2	90	—	5	5	10
	0.2	.0014	1	80	10	—	10	10
	0.1	.0007	2	75	15	5	5	10
Methyl- $\alpha$ -naphthyl- amine	1.0	.0064	2	—	—	10	90	100
	0.75	.0048	2	—	—	11	89	100
$C_{10}H_7NH(CH_3)$	0.5	.0032	2	20	5	25	50	75
	0.35	.0021	1	60	—	—	40	40
Dimethyl- $\alpha$ - naphthylamine	1.0	.0058	1	—	—	—	100	100
	0.75	.0044	1	—	—	—	100	100
$C_{10}H_7N(CH_3)_2$	0.5	.0029	2	25	10	10	55	65
	0.35	.002	1	60	20	—	20	20
Diethyl- $\alpha$ -naphthyl- amine	1.0	.005	1	—	—	—	100	100
	0.75	.0037	2	—	5	—	95	95
$C_{10}H_7N(C_2H_5)_2$	0.5	.0025	3	40.5	4	7.5	48	55.5
	0.25	.0013	2	70	—	—	30	30
Phenyl- $\alpha$ -naphthyl- amine	1.0	.0046	2	—	—	40	60	100
	0.75	.0034	2	5	—	40	55	95
$C_{10}H_7NH-C_6H_5$	0.5	.0023	2	5	10	40	45	85
	0.35	.0017	1	30	—	60	10	70
	0.25	.0011	1	50	20	—	30	30

Ethyl- $\alpha$ -naphthylamine gave irregular results but a mortality of 90–100 per cent. was shown at concentrations between 1 and 2 per cent. Amyl- $\alpha$ -naphthylamine (containing some di-derivative) and nitroso- $\alpha$ -naphthylamine (commercial) secured a mortality of 80–100 per cent. at concentrations of 0.5–0.75 per cent. Di- and tetrahydro- $\alpha$ -naphthylamine (*ar*), dihydro-dimethyl- $\alpha$ -naphthylamine gave mortalities between 80 and 100 per cent. at concentrations between 0.75 and 1.0 per cent.

$\beta$ -Naphthylamine and its derivatives were less toxic than  $\alpha$ -naphthylamine and its derivatives.  $\beta$ -Naphthylamine, dimethyl- $\beta$ -naphthylamine, phenyl- $\beta$ -naphthylamine, tetrahydro- $\beta$ -naphthylamine (*ac*) were not completely toxic at concentrations of 1.0 per cent.

1 : 2-naphthalene-diamine was not toxic at a concentration of 1.0 per cent.

\* Benzene used as a solvent at the rate of 2 c.c. per cent. of the spray fluid for the highest concentration (1 per cent.  $\alpha$ -naphthylamine), and in proportion at the lower concentrations.

Figures in Table III show that  $\alpha$ -naphthylamine is rather more toxic than  $\beta$ -naphthylamine.

The points expressing the toxicities of a number of derivatives such as methyl-, dimethyl-, ethyl- and diethyl- $\alpha$ -naphthylamines fall within the shaded area of Diagram 3. All the  $\beta$ -naphthylamine derivatives tested were less toxic than the corresponding  $\alpha$ -compounds.

$\alpha$ -Naphthylamine is also more toxic, weight for weight and mole for mole, than aniline; in general, however, radicals which when substituted in the aniline molecule produce distinct increases in toxic action, have a less marked effect when substituted in  $\alpha$ -naphthylamine.  $\alpha$ -Naphthylamine was tested both as a fine suspension and as an emulsion of its benzene solution in saponin and water. The preparation of a fine suspension of  $\alpha$ -naphthylamine in 1 per cent. saponin solution is a matter of some difficulty, and it is usually unstable owing to aggregation of the particles and the growth of crystals. The finely suspended solid was more toxic than the emulsion of the benzene solution in saponin solution, the figures for the former falling outside the left margin of the shaded area in Diagram 3 and those for the latter lying on the right. The results for the fine suspension are based, however, on one experiment only, as, except on one occasion, we were unable to maintain the suspension for a sufficient length of time to carry out the trials.

As in the case of the corresponding aniline derivatives, phenyl- $\alpha$ -naphthylamine and nitro- $\alpha$ -naphthylamine<sup>1</sup> were more toxic than the other  $\alpha$ -naphthylamine derivatives tested. The results are expressed on Diagram 3 by the dotted curve.

For purposes of comparison the results obtained with a few of the more toxic compounds of aniline and  $\alpha$ -naphthylamine are plotted together in Diagram 4.

Diphenylamine is appreciably more toxic than phenyl- $\alpha$ -naphthylamine, but nitraniline is rather more toxic than the crude sample of nitro- $\alpha$ -naphthylamine, when comparisons are made between concentrations on a gram percentage basis. Diagram 4 shows that, mole for mole, however, one curve represents with a fair degree of accuracy the toxicities of all these derivatives.  $\alpha$ -Naphthylamine itself is more toxic than aniline, both weight for weight and mole for mole: the introduction of the naphthyl group into the ammonia radical ( $\text{NH}_2$ )<sup>2</sup> affects toxicity more than the introduction of a phenyl group. The substitution of a

<sup>1</sup> The nitro-naphthylamine was a technical product, which could not be purified readily by recrystallisation.

<sup>2</sup> Ammonia itself in solution is only very slightly toxic.

phenyl nucleus, however, in the amino group of aniline increases toxicity proportionally to a greater extent than its substitution in the amino group of  $\alpha$ -naphthylamine.

Looking at these relationships between toxicity and structure from the standpoint of substitution in the respective hydrocarbon nuclei, we find that the introduction of the amine group into benzene or into naphthalene materially increases toxicity; but the influence of the amino group does not seem to be additive, since the introduction of a second  $\text{NH}_2$  does not give rise to any great increase in toxicity; indeed,

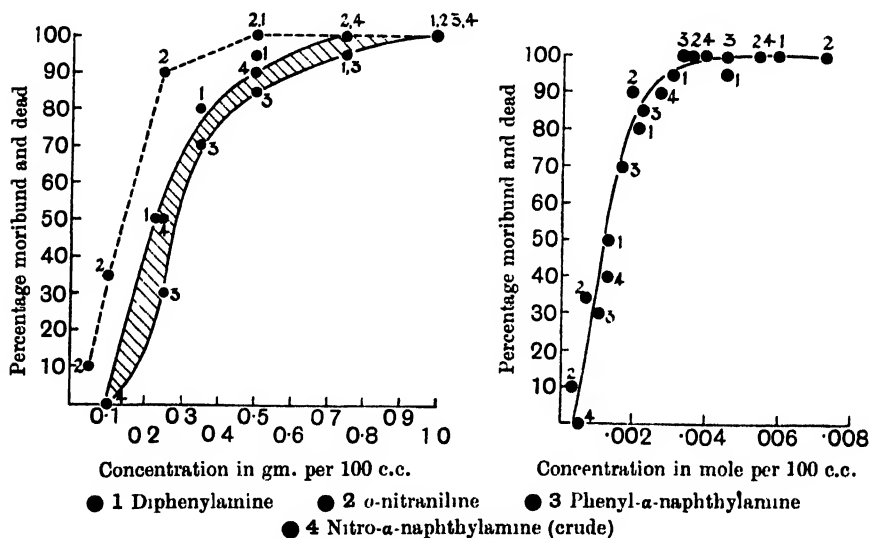


Diagram 4. Toxicities of diphenylamine, *o*-nitraniline, phenyl- $\alpha$ -naphthylamine and nitro- $\alpha$ -naphthylamine (crude) to *Aphis rumicis*

the  $\alpha$ - $\beta$ -diamine of naphthalene is less toxic than  $\alpha$ -naphthylamine. The  $\text{NH}_2$  group in the aromatic amines may act by increasing the affinity for oxygen and there may be an optimum degree of reactivity and, if a compound absorbs oxygen too rapidly, its toxic action may be destroyed before reaching a vital point of the insect. Thus the diamines which are less stable than the monamines may well show a less toxic action because they are oxidised to innocuous derivatives shortly after spraying.

Hydrogenation of the naphthylamine molecule does not produce any marked increase in toxicity; *ar*-dihydro and *ar*-tetrahydro- $\alpha$ -naphthylamine and the *ac*-tetrahydro- $\beta$ -naphthylamine are not more toxic than  $\alpha$ -naphthylamine.

*Nitrogen-heterocyclic Compounds.*

This group of compounds is of considerable importance from an insecticidal point of view. It includes nicotine; and in recent years the dipyriddyis prepared by C. R. Smith<sup>(13)</sup> have been shown by Richardson<sup>(14)</sup> to have a high potency as contact insecticides; pyridine and its homologues have also occasionally been employed for this purpose. An examination was therefore undertaken of the toxic properties of the more commonly occurring members of this series.

*Nicotine* ( $\beta$ -pyridyl- $\alpha$ -N-methylpyrrolidine). As a contact insecticide, nicotine has been in common use for many years and is now regarded as the standard material for this type of work; for that reason we refer to it first among the heterocyclic compounds.

Much work has been done by various investigators on the physiological action of nicotine upon the higher animals, and it has been shown to have certain characteristic effects on the nervous system. McIndoo<sup>(15)</sup> showed that spray solutions of nicotine neither pass into the tracheae nor penetrate the integuments of insects, whereas the vapour does so and is there condensed; it is however found distributed to all the tissues. In insects, as well as in the higher animals, nicotine kills by paralysis of the nervous system, travelling along the central cord from the abdomen to the brain. McIndoo states that it gives rise to the same physiological changes as those caused by deprivation of oxygen.

It was convenient in the earlier part of this work to use pure nicotine as a standard of comparison and a large number of tests have been

Table IV.

*Toxicity of nicotine to A. rumicis.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration		No. of insects	N %	S %	M %	D %	M and D %	M and D allowing for control
	Gm. per 100 c.c.	Mole per 100 c.c.							
Nicotine	.25	.0015	108	0.9	—	1.8	97.2	99.0	99.0
	.2	.0012	210	1.0	0.5	5.7	92.9	98.6	98.5
	.15	.0009	143	7.7	2.1	9.1	81.1	90.2	89.8
	.1	.0006	170	7.1	2.9	12.9	77.1	90.0	89.6
	.08	.0005	149	6.0	12.1	22.8	59.1	81.9	81.1
	.06	.00035	145	19.8	4.7	17.1	57.4	74.5	73.4
	.04	.00025	148	38.5	11.5	11.5	38.5	50.0	47.8
	.02	.0001	143	76.9	2.8	9.1	11.2	20.3	16.8
	.01	.00006	210	79.5	3.3	4.3	12.9	17.3	13.7
	.005	.00003	145	91.7	0.7	1.4	6.2	7.6	3.6
	.0025	.00001	148	93.9	—	1.4	4.7	6.1	2.0
Control	—	—	142	95.1	0.7	0.7	3.5	4.2	—



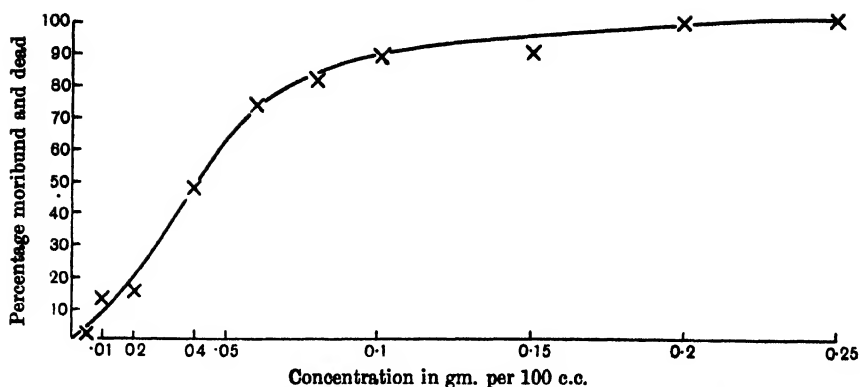


Diagram 5. Toxicity of nicotine to *Aphis rumicis* (allowing for controls).  
Average for three years' results.

carried out in the course of our experiments, the results being set out in Table IV and Diagram 5.

These figures are the average of the results obtained during three years, the total number of insects used at each concentration being over 100. Under the conditions of the experiments, in which the insects used were all at the same stage (adult apterous agamic females), nicotine is probably less effective than if aphides, including all stages of development, were used. There was some evidence, during the course of the experiments, that external meteorological conditions had an effect upon toxicity, but the data on this point are not sufficiently full to warrant discussion at this stage.

Our results indicated that, on the average, a concentration of about 0.1 per cent. of this alkaloid killed 90–100 per cent. of the adult insects which is in fairly close agreement with the opinion of Pickering<sup>(16)</sup> that a concentration of 0.075 per cent. was effective against aphids, apple psylla, etc. In small scale field-trials on beans heavily infested with all stages of *A. rumicis*, we have found that, in general, lower concentrations of nicotine than the above established a good control. Thus, with soft soap as a spreader, a concentration of 0.025 per cent. gave good control, 0.05 per cent. good to complete control, and 0.1 per cent. complete control.

*Pyrrole, pyrrolidine, pyridine and piperidine.* The results obtained for these compounds are given along with the other members of this series tested, in Table V.

They are of special interest owing to their close relationship to nicotine.

Table V.

*Toxicities to A. rumicis of some N-heterocyclic compounds.*

[N=not affected; S=slightly affected; M=moribund; D=dead.]


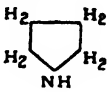


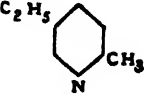
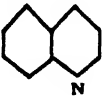

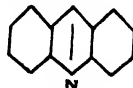
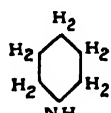
Substance	Concentration		No. of tests	N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.						
Pyrrole								
								
								Shows an anaesthetic action. Not materially toxic at a concentration of 10.0 %
Pyrrolidine								
								
	3.5-2.5	.049-.035	2	—	—	5	95	100
	1.0	.014	1	—	—	—	100	100
	0.75	.011	1	60	10	10	20	30
	0.5	.007	1	100	—	—	—	—
Pyridine								
								
	10.0	.13	2	—	—	50	50	100
	7.5	.095	1	—	—	100	—	100
	5.0	.063	2	50	—	35	15	50
	2.5	.031	2	58	10.5	21	10.5	31.5
	1.0	.013	2	65	10	20	5	25
	0.75	.0095	1	90	—	10	—	10
								Has an anaesthetic effect
$\alpha$ -Picoline								
								
	10.0	.11	1	—	—	40	60	100
	7.5	.081	1	—	—	20	80	100
	5.0	.054	1	—	20	50	30	80
	2.5	.027	1	60	20	—	20	20
Lutidine (CH <sub>3</sub> ) <sub>2</sub> C <sub>6</sub> H <sub>7</sub> N								
	10.0-5.0	.083-.047	3	—	—	—	—	100
	2.5	.023	2	15	—	10	75	85
	1.0	.0093	2	30	—	10	60	70
	0.75	.007	1	90	—	—	10	10
	0.5	.0047	2	95	—	5	—	5
Collidine (2-methyl-5-ethyl- pyridine)								
								
	5.0	.041	1	—	—	—	100	100
	3.5	.031	1	—	40	40	20	60
	2.5	.021	1	20	60	10	10	20
	1.0	.0083	1	70	10	—	20	20
Quinoline								
								
	5.0-2.5	.039-.019	3	—	—	—	—	100
	1.5	.011	1	—	—	40	60	100
	1.0	.0077	3	6.5	6.5	30	57	87
	0.75	.0058	2	25	5	45	25	70
	0.5	.0039	3	14	18	3.5	64.5	68
	0.25	.0019	3	57	10	7	26	33
	0.1	.00077	2	95	—	—	5	5

Table V (continued)

[N = not affected; S = slightly affected; M = moribund; D = dead.]

		Concentration		No. of tests	N %	S %	M %	D %	M and D %
Substance		Gm. per 100 c.c.	Mole per 100 c.c.						
	Isoquinoline	2.5	0.019	1	—	—	10	90	100
		1.5	0.011	1	—	—	—	100	100
		1.0	0.0077	2	5	—	20	75	95
		0.75	0.0058	2	15	5	30	50	80
		0.5	0.0039	2	60	10	10	20	30
		0.25	0.0019	2	90	10	—	—	—
	Acridine	1.0	0.0056	1	—	10	50	40	90
		0.75	0.0042	1	—	—	100	—	100
		0.5	0.0028	1	—	10	70	20	90
		0.25	0.0014	1	10	10	80	—	80
		0.1	0.00056	1	80	—	20	—	20
		0.05	0.00028	1	90	10	—	—	—
	Piperidine	5.0-3.5	0.059-0.041	2	—	—	25	75	100
		2.5	0.029	2	—	—	45	55	100
		1.0	0.012	2	40	25	5	30	35
		0.75	0.0088	1	90	—	—	10	10
		0.5	0.0059	2	70	15	10	5	15
		0.25	0.0029	2	90	10	—	—	—
Benzylpyridine* distilling 276°- 278° C.		2.5-1.0	0.015-0.0059	2	—	—	—	—	100
		0.5	0.003	3	—	—	13	87	100
		0.35	0.002	2	5	5	10	80	90
		0.25	0.0015	1	—	—	70	30	100
		0.2	0.0012	2	35	—	10	55	65
		0.1	0.00059	2	45	—	20	35	55
		0.075	0.00044	1	60	—	—	40	40
		0.05	0.0003	1	80	—	—	20	20
Crude benzylpyridine distilling in steam		0.5	—	2	—	—	5	95	100
		0.35	—	2	—	—	15	85	100
		0.2	—	2	—	—	25	75	100
		0.1	—	2	15	25	20	40	60
		0.075	—	2	20	25	10	45	55
		0.05	—	2	5	35	25	35	60
		0.025	—	1	20	40	20	20	40
Pyridine-N-benzyl chloride C <sub>5</sub> H <sub>5</sub> NC <sub>7</sub> H <sub>7</sub> Cl		5.0-2.5	—	2	—	—	—	100	100
		1.0	—	3	—	—	42	58	100
		0.75	—	2	30	—	35	35	70
		0.5	—	2	30	5	20	45	65
		0.35	—	1	40	40	20	—	20

N-methyl- and isopropyl-piperidines were of the same order of toxicity as piperidine. Isoamyl- and benzyl-piperidine were slightly more toxic than piperidine. Quinaldine and tetrahydroquinaldine secured a mortality between 80 and 100 per cent. at concentrations of 0.5-1.0 per cent. 2:4-Dimethylquinoline, ehinosol sulphate and base, and acriflavine were not completely toxic at 1.0 per cent. The choline bases of pyridine and their hydrochlorides secured a mortality of 80-100 per cent. at concentrations of 2.5 per cent. Triacetaminine was not completely toxic at a concentration of 2.5 per cent.  $\alpha$ -amino-pyridine and 2:6-diamino-pyridine were not toxic at concentrations of 1 gm. per 100 c.c.

\* The fractions of benzyl pyridine distilling from 278° to 304° C. differed little in toxicity from the fraction distilling 276° to 278° C.

Neither pyrrole not pyridine have any marked toxic action. Pyrrole shows an anaesthetic effect on *A. rumicis* and at such considerable concentrations as 10.0 per cent. the majority of insects sprayed recovered. We found pyridine to be slightly more poisonous than pyrrole, nevertheless its toxicity is so slight as to render it almost worthless as an insecticide. Brunton and Tunnicliffe(18) have shown that although pyridine has some slight effect upon the sensory apparatus, respiration and heart action of frogs, it can be regarded as comparatively non-poisonous. It is curious that it should have been found(17) to be highly toxic in the vapour phase to certain mites and their eggs, to which it seems a highly specific poison.

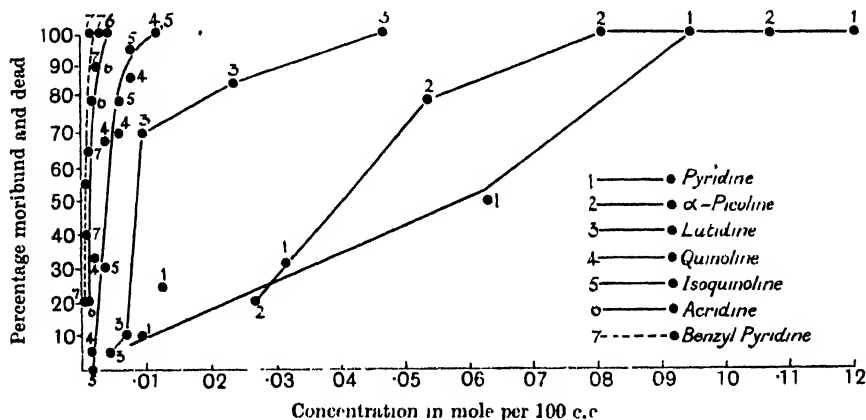


Diagram 6. Toxicity of certain heterocyclic derivatives to *Aphis rumicis*.

The effect of hydrogenation on the toxicity of both pyrrole and pyridine is considerable, pyrrolidine and piperidine being found to be relatively highly toxic when compared with their parent substances. This may be due to increased reactivity and a consequent increased effect upon the nervous system. It has been shown (cf. Spiegel(19)) that pyrrolidine and piperidine have a paralytic action on both central and peripheral nerves. Both are however much less toxic to insects than nicotine, which undoubtedly owes its high potency to its molecular make-up taken as a whole; the attachment of the pyrrolidine nucleus in the  $\beta$ -position of the pyridine ring, and the presence of an asymmetric carbon atom may be significant.

The results obtained with pyridine and various other members of this group, ranging from pyridine to acridine, are expressed in Table V and Diagram 6. Certain other compounds such as the naphthacridines

were prepared, but were too slightly soluble to be tested in any thorough way. They did not appear to possess any marked toxic properties.

In Diagram 6, the concentrations are plotted as fractions of a gram-molecule per 100 c.c. against the percentage of moribund and dead. There is a steady increase in toxicity as the molecular weight increases: pyridine < picoline < lutidine < quinoline and isoquinoline < acridine. We were unable to secure a sample of trimethylpyridine (collidine); its isomeride ethylmethylpyridine was slightly less toxic than lutidine.

M'Kendrick and Dewar<sup>(20)</sup> have shown that with various types of animals, the physiological action on the sensory nerves is augmented by the introduction of aliphatic side chains into compounds of this group, and that in the series pyridine, picoline, lutidine, etc., there is an increase in the effect in passing up the series. In their action upon insects the same order is observed. Whether this is due to an increase in molecular weight, or to change in some physical property in correlation with the increased bulk of the molecule, has not up to the present been ascertained.

The toxicities of quinoline and isoquinoline are identical and apparently the points at which the pyridine and benzene nuclei are fused in these compounds are unimportant in determining toxicity to insects.

The substitution of a benzyl group has a pronounced effect on the toxicity of pyridine. Benzylpyridine prepared by the method of Tschitschibabin<sup>(21)</sup>, and apparently a mixture of the  $\alpha$ - and  $\beta$ -derivatives, was more toxic than any other pyridine derivative tested, except nicotine. Concentrations of about 0.2 per cent. killed 100 per cent. of the insects.

The toxicity of the pyridine ring can therefore be increased by hydrogenation, by the introduction of side chains and by fusion with one or more benzenoid rings. A fourth method is the attachment of a second pyridine ring as in the dipyridyls; these compounds are not dealt with in this paper but the work of Richardson and Smith<sup>(13, 14)</sup> shows them to be much more toxic than pyridine<sup>1</sup>.

*The Dependence of the Toxic Values of Insecticides on the  
Phase in which they are applied.*

Tattersfield and Roberts<sup>(22)</sup> tested the insecticidal values of a few of the compounds dealt with in this paper, using wire-worms as the test-subject and applying the insecticide in the vapour phase in closed flasks

<sup>1</sup> Since this paper was handed in for publication, a full account of the work of these authors on the dipyridyls has appeared (*Jour. Agr. Res.* 1926, **33**, 597), in which the high toxicity of certain of these derivatives to insects is demonstrated.

under standard conditions. Although these conditions differ widely from those employed in our spraying technique, it is of some interest to make a general comparison of the kind of effects obtained, although it cannot be put on a quantitative basis.

Amongst the inorganic compounds, ammonia gas was, relative to other compounds, moderately toxic to wireworms, when its concentration was expressed in fractions of a gram-molecule per litre of air, and highly toxic when expressed in grams per litre of air. As a spray fluid, ammonium hydroxide was scarcely toxic to aphides at a concentration of 9 gm. per 100 c.c. Although the amount of ammonia falling on any individual aphid can only be very minute and will evaporate at a fairly rapid rate, it is of interest that, to an insect of the type of *A. rumicis*, ammonia should be comparatively harmless when applied in solution, whereas when applied as gas, it should have so profound an effect upon wireworms, commonly regarded as highly resistant insects.

Benzene, toluene, xylene are not active as insecticides(3) but the toxicity increases with molecular weight; this agrees with the type of results obtained by Tattersfield and Roberts for the effect of these substances in the vapour phase. There is, moreover, both in the liquid and vapour phases a definite increase in toxicity on substituting an  $\text{NH}_2$  group in these rings. There is also in both cases an increase in toxicity produced by methylating the amino group of aniline.

The only other instance common to both investigations, in which direct comparisons can be made, is the case of pyridine, found by us to have a toxicity to *A. rumicis* of a very low order when used as a spray fluid and by Tattersfield and Roberts to be only moderately toxic as vapour to wireworms. As already noted however pyridine in the vapour phase has apparently a specific toxic action to mites and their eggs.

$\alpha$ -Naphthylamine and diphenylamine were non-toxic under the conditions of the experiments of Tattersfield and Roberts, whereas as sprays they are moderately toxic. Presumably the toxicity of these compounds in the vapour phase was limited by low volatility, and a prolonged exposure of the insects might have changed the category in which these compounds were placed.

#### *Plot experiments.*

A few substances found toxic in the laboratory experiments were tried on a larger scale on plots of broad beans badly infested with *A. rumicis*. Wetting and spreading was ensured by making up each spray fluid with 0.35 per cent. soft soap solution.

Tetramethylammonium chloride and sulphate were both tried under these conditions; they were completely effective in controlling the aphides at a concentration of 0.35 per cent. of the base, and there was no sign of injury to the plants. The sulphate also gave good results at a concentration of 0.125 per cent. of the base.

Some of the aromatic amines were also tried.  $\alpha$ -Naphthylamine and mono-methyl- $\alpha$ -naphthylamine destroyed the aphides when used at concentrations of 0.4 per cent. and above, but also caused considerable damage and staining of the foliage. The latter effect was not shown by dimethyl- $\alpha$ -naphthylamine which however was slightly less effective as an insecticide.

The only pyridine derivative tested in this manner was benzyl-pyridine. The results bore out completely the figures obtained in the laboratory experiments, all the insects being killed by a spray fluid containing 0.1 per cent. of this compound. Moreover, no damage whatever was caused to the plants. Benzyl-pyridine is an oil without disagreeable properties and, if it could be prepared reasonably cheaply, it might find some use as a contact insecticide. Its high toxicity warrants further work on the subject.

#### SUMMARY.

1. The toxicities to *Aphis rumicis* of certain aliphatic and aromatic amines and of some of the simpler nitrogen-heterocyclic derivatives have been quantitatively determined.

2. Tetramethylammonium hydrate and chloride are more toxic than the corresponding tetraethylammonium compounds. This is in keeping with the findings of Dale and his co-workers who have shown that tetramethylammonium has certain physiological effects similar to those of nicotine which are not shown by tetraethylammonium.

3. The aromatic amines, on the whole, show little insecticidal action. Aniline and most of the aliphatic anilines are only slightly toxic to *A. rumicis*. The substitution of aromatic groups in the amino group of aniline increases toxicity more than the substitution of aliphatic groups. There are interesting relationships in regard to toxicity among these compounds. For example, the following orders of toxicity were noted:

Phenylamine (aniline) < diphenylamine > triphenylamine.

Phenylamine (aniline) < benzyaniline > dibenzyaniline.

Benzylamine < dibenzylamine > tribenzylamine.

4. *o*-Nitraniline is one of the most toxic of the aniline derivatives.

5.  $\alpha$ -Naphthylamine is more toxic than aniline. Substitution of various radicals in the amino group of aniline has a greater effect on the toxicity of the resulting compound than substitution of the same radicals in  $\alpha$ -naphthylamine.  $\alpha$ -Naphthylamine derivatives are more toxic than the corresponding  $\beta$ -derivatives.

6. Among the heterocyclic compounds, nicotine is highly poisonous to *A. rumicis*. The heterocyclic rings constituting the molecule of nicotine are much less toxic than nicotine itself; pyrrole and pyridine show comparatively slight insecticidal action. The order of toxicity of the simpler N-heterocyclic compounds runs:

Pyrrole < pyridine < picoline < lutidine < quinoline and isoquinoline < acridine.

7. Hydrogenation of pyridine and pyrrole increases their toxicity; piperidine is more toxic than pyridine and pyrrolidine than pyrrole.

8. Benzyl-pyridine is the most toxic pyridine derivative tested.

#### LITERATURE CITED.

- (1) TATTERSFIELD, F. and MORRIS, H. M. (1924). *Bull. Ent. Res.* xiv, 223.
- (2) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* xii, 61.
- (3) ——— (1925). *Ibid.* xii, 218.
- (4) BROWN, A., CRUM and FRASER, T. R. (1869). *Proc. Roy. Soc. Edin.* vi, 556.
- (5) BARGER, G. and DALE, H. H. (1910). *Journ. Physiol.* xli, 19.
- (6) BURN, J. H. and DALE, H. H. (1915). *Journ. Pharm. and Exptl. Therap.* vi, No. 4, 417.
- (7) RICHARDSON, C. H. and SMITH, C. R. (1923). *U. S. Dept. Agric. Bull.* 1160.
- (8) OVERTON, E. (1901). *Studien über die Narkose.* Jena.
- (9) MEYER, H. (1899). *Arch. Expt. Path. Pharm.* xlii, 109.  
—— (1901). *Ibid.* xlv, 337.
- (10) HARVEY, E. N. *Carnegie Inst. of Washington*, vi, 133.
- (11) FLUSIN, G. (1900). *Compt. Rend.* cxxxi, 1308.
- (12) DALE, H. H. (1920). *Johns Hopkins Hospital Bull.* xxxi, No. 356, 22.
- (13) SMITH, C. R. (1924). *Journ. Amer. Chem. Soc.* xlv, 414.
- (14) RICHARDSON, C. H. (1925). *Journ. Econ. Ent.* xviii, 281. (Abstract only.)
- (15) MCINDOO, N. E. (1916). *Journ. Agric. Res.* vii, 89.
- (16) DUKE OF BEDFORD and PICKERING, S. U. (1919). *Science and Fruit Growing*, 147.
- (17) JEWSON, S. T. and TATTERSFIELD, F. (1922). *Ann. App. Biol.* ix, 213.
- (18) BRUNTON, T. L. and TUNNICLIFFE, F. W. *Journ. Physiol.* xvii, 272.
- (19) SPIEGEL, L. (1915). *Chemical Constitution and Physiological Action*, p. 94.
- (20) M'KENDRICK, J. G. and DEWAR, J. (1874). *Proc. Roy. Soc.* xxii, 432.
- (21) TSCHITSCHIBABIN, A. E. and RIUMSCHIN, P. F. (1915). *Journ. Russ. Phys. Chem. Soc.* xlvii, 1297. [*Journ. Chem. Soc.* (1915), cviii, A 1, 993.]
- (22) TATTERSFIELD, F. and ROBERTS, A. W. R. (1920). *Journ. Agric. Sci.* x, 199.

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## ON THE CONTROL OF GLASSHOUSE INSECTS WITH CALCIUM CYANIDE

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DURING the past few years, as pointed out by Speyer and Owen(4), efforts have been made in America and Germany to develop a means of generating hydrocyanic acid gas by a method simpler than that of jar cyaniding, which has certain attendant disadvantages, including the necessity for separate weighings and measurings of sodium cyanide, sulphuric acid and water, the need for numbers of jars in large commercial houses, the labour involved and the limitations resulting from weather conditions. As a result of investigations in the United States(1), calcium cyanide containing 40-50 per cent.  $\text{Ca}(\text{CN})_2$  was manufactured, and after tests by Weigel(7) and others, this material has become widely used as a cheap and convenient source of hydrocyanic acid gas. Several grades of calcium cyanide, including dust and fine and coarse granules, are now available; the fine granular form recommended for glasshouse fumigation is like sharp sand and resembles basic slag in colour. With calcium cyanide no acid is required to generate the gas, and no mixing is necessary, for on exposure it slowly reacts to the moisture in the atmosphere and hydrocyanic acid is given off. Under glasshouse conditions the reaction continues for several hours and thus renders possible a long exposure with a low concentration, the type of fumigation recommended by Lloyd(2) for the control of the greenhouse white fly, *Trialeurodes vaporariorum* Westw. In May 1925, calcium cyanide was introduced into Great Britain, and since that date a large number of fumigations have been carried out in various parts of England(3), using this material as a source of hydrocyanic acid for the control of white fly, aphides and thrips, and the following is an account of the results so far obtained. I am indebted to Mr C. T. N. Wakely, B.Sc., and Mr A. Turner, A.R.C.Sc.I., N.D.H., for their help in conducting the fumigations, and to Mr F. Laing of the British Museum and Dr J. Davidson of Rothamsted, for identifying certain of the insects dealt with.

*Methods.*

In conducting the fumigations herein discussed, the cubic capacity of the glasshouse was first determined, and the calcium cyanide was measured by means of a graduated distributing funnel. The funnel used was provided with a lid and a tap, the former to prevent the escape of the fumes during application, and the latter to provide an easy means of running the calcium cyanide off. The fumigant was applied along the paths of the house and all ventilators closed and doors locked for the night. Next day the house was opened up early and after an hour or so examination for results was made.

In recording the results, where no live insects of the species against which the fumigation was conducted, could be found after careful search, 100 per cent. kill was recorded; where, in a heavy infestation, only odd active specimens could be found 90 per cent. kill was recorded. Where the kill was only partial, estimations were made in several parts of the glasshouse and the results averaged. When the pest was present in small numbers only, counts were made and the percentage figure obtained from them. In the case of fumigations for the control of white fly, where a number of fumigations were carried out at the same dosage, the lowest and highest estimated percentage kill figures are given to indicate the range of control obtained. In the case of the aphid control fumigations, the per cent. kill figures represent the average estimated kills from the fumigations at each dosage. While absolute accuracy is not claimed, the figures serve to indicate the degree of control obtained, particularly when taken in conjunction with the number of fumigations carried out.

*Experiments on the control of white fly (Trialeurodes  
vaporariorum Westw.).*

Fifty-four fumigations were conducted in glasshouses in various parts of Great Britain and under a variety of conditions, and in twenty-seven cases 90 per cent. kill or over was obtained on the adult insects. The following table indicates the dosages employed and the conditions as to temperature and humidity obtaining at the time of fumigation.

Plants fumigated included chrysanthemum, azalea, runner bean, cucumber, freesia, tomato and arum. In most cases the temperature varied between 55° and 70° F. at the commencement of fumigation and the relative humidity varied from 70-95 per cent. The lower dosages were employed when fumigating young plants such as seedling tomatoes

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No. of fumigations	Dosage per 1000 cu. ft. (oz.)	Greenhouse temperature	Relative humidity %	Control (kill) %
2	$\frac{1}{2}$	62-71° F.	—	60
2	$\frac{1}{2}$	60-67° F.	90	70-80
4	$\frac{1}{2}$	54-70° F.	72-95	80
1	$\frac{1}{2}$	64° F.	79	80
11	$\frac{1}{2}$	58-67° F.	71-87	90-100
17	$\frac{1}{2}$	56-68° F.	84-87	50-100
11	$\frac{3}{4}$	52-70° F.	—	50-100
2	$\frac{3}{4}$	58-70° F.	—	100
4	$\frac{3}{4}$	59-77° F.	—	100

and cucumbers. In spring, when plants are usually soft and tender, they can only be fumigated with a low dosage, but under suitable conditions dosages as low as  $\frac{1}{2}$  oz. per 1000 cu. ft. were found to keep the white fly in satisfactory check. The  $\frac{1}{2}$  oz. dosage was employed for plants such as tomatoes in normal growth;  $\frac{1}{2}$  oz. for chrysanthemums, and the higher dosages from  $\frac{1}{2}$  oz. to  $\frac{3}{4}$  oz. for mature tomatoes towards the end of the season, under abnormally dry conditions. Increased dosages were, of course, necessary in leaky houses. The moisture factor was variable; under very dry conditions it was necessary to damp the paths an hour or two before fumigation; in other cases the paths were so damp that satisfactory fumigations were not obtained until the cyanide was sprinkled on dry boards. Under average conditions in glasshouses, however, sufficient moisture was present for the satisfactory evolution of hydrocyanic acid gas from the calcium cyanide. Previous to fumigating open tanks were usually covered with sacks or boards. The calcium cyanide was distributed by means of a funnel provided with a tap to allow for the regulation of the flow, thus permitting even distribution throughout the greenhouses. Local conditions influenced results to a considerable extent, leaky houses, excess of moisture on the paths, low temperatures and high winds often being responsible for unsatisfactory results.

### *Experiments on the control of aphides.*

Several species of aphides cause considerable injury in glasshouses, and in the course of these investigations at least five species have been encountered in various parts of England, all being more or less common and generally distributed. The following table lists the species and fumigating conditions, together with the dosages used:

	Dosage per 1000 cu. ft. (oz.)	Greenhouse temperature	Relative humidity %	No. of fumiga- tions	Results (kill) %
<i>Myzus persicae</i> Sulz.	$\frac{1}{8}$	71° F.	73	1	80
	$\frac{1}{4}$	62° F.	79	2	70
	$\frac{1}{2}$	58-62° F.	83-89	3	90
	$\frac{1}{4}$	52-66° F.	83-94	8	98
	$\frac{1}{8}$	60° F.	89	1	90
	$\frac{1}{2}$	52° F.	—	1	100
<i>Macrosiphum gei</i> Koch.	$\frac{1}{8}$	60° F.	86	2	75
	$\frac{1}{4}$	65° F.	—	1	60
<i>M. lineatum</i> V. d. Goot.	$\frac{1}{2}$	55° F.	88	2	50
<i>Myzus circumflexus</i> Buck.	$\frac{1}{4}$	53-65° F.	83-89	5	78
	$\frac{1}{8}$	59° F.	68	1	80
	$\frac{1}{2}$	53-55° F.	88-90	2	90
<i>Macrosiphoniella sanborni</i> Gillette.	$\frac{1}{4}$	53° F.	—	1	100
	$\frac{1}{2}$	53-55° F.	—	2	100
<i>Aphis rumicis</i> L.	$\frac{1}{8}$	59° F.	89	1	90

When used under suitable conditions, with the temperature between 60° and 70° F., and relative humidity of 80-90 per cent., effective control was generally obtained with a dosage of  $\frac{1}{8}$ - $\frac{1}{2}$  oz. of calcium cyanide per 1000 cu. ft. In practice it was found most satisfactory to employ a low dosage and carry out two or three fumigations at about weekly intervals. *Myzus persicae*, which lives and breeds throughout the year under glasshouse conditions and infests a great variety of plants, is the species most commonly met with under glass. *Myzus circumflexus* occurs commonly on arums and is occasionally found on tulips, cyclamen and freesias, though most glasshouse plants may be attacked. According to Theobald (5), this species increases most rapidly from February to May, although it is found all the year under glass. *Macrosiphum gei* Koch. (*solanifolii* Ashm.) occurred on tulip in the fumigations recorded above. This species is known to attack sprouting potatoes (6). *Macrosiphum lineatum* V. d. Goot occurred commonly on chrysanthemums at Worthing and Cambridge in November 1926. *Macrosiphoniella sanborni* occurs on chrysanthemums out of doors from September to November and throughout the winter under glass. Under ordinary greenhouse conditions it is found that the species is easily controlled at a dosage of  $\frac{1}{2}$  oz. per 1000 cu. ft., whereas the other species under similar conditions may need a higher dosage.

#### *Experiments on thrips.*

During the course of these investigations four species of thrips have been dealt with: *Heliothrips bicinctus* Bagn. on smilax, *Thrips tabaci* Lind. on smilax, arums and carnations, *Parthenothrips dracaenae* Heeg.

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on Kentias and other palms and *H. haemorrhoidalis* Bouché. on tomatoes, arums and scolopendrium fern. Fumigations with calcium cyanide were found to be successful in killing adult thrips, but failed to destroy the immature forms. In the case of attack by *T. tabaci* on carnations, the insects, especially the adults, infest the opening blooms and, where vast numbers are found deep down amongst the bases of the petals and within the sepals, their feeding may almost bleach the petals. The hydrocyanic acid gas penetrated the flowers well and was found to destroy thrips even in such locations. *H. haemorrhoidalis* occurs fairly commonly on arums, and in one case in Guernsey infestation was so severe in a house of tomatoes as to make the grower consider destroying the plants and re-planting. Periodic fumigations were found to destroy the adults and gradually reduce infestation, and the plants commenced making cleaner growth shortly after the first fumigation. The control of both species on arums was effected with comparative ease, but with *H. bicinctus* on smilax four fumigations were necessary to effect satisfactory control.

The following table lists the dosages employed and the conditions obtaining:

Species of thrips	Plant	Dosage per 1000 cu. ft. (oz.)	Greenhouse temperature	Relative humidity %	Result (kill) %
<i>Thrips tabaci</i>	Carnation	$\frac{1}{2}$	60° F.	—	10
	"	$\frac{1}{2}$	62° F.	—	*
	"	$\frac{1}{2}$	59° F.	—	65
<i>Heliothrips haemorrhoidalis</i>	Tomatoes	$\frac{1}{2}$	59–60° F.	72–73	30–40
	Arums	$\frac{1}{2}$	60° F.	89	70
<i>H. bicinctus</i>	Smilax	$\frac{1}{2}$	59° F.	97	10–20†
<i>Parthenothrips dracaenae</i>	Kentia palms	1½	60° F.	—	90†

\* Partial control. † Many stupefied but revived later. ‡ Nymphs apparently affected.

### *Calcium cyanide fumigations and mealy bug control.*

Though no experimental fumigations have been carried out where definite counts for mortality give an accurate idea of the amount of control obtained, the following experiments in Guernsey indicate that calcium cyanide will give satisfactory control of mealy bug on tomatoes and vines. Tomatoes grown in a glasshouse where *Pseudococcus maritimus* Erh. occurs as a pest each year, were found by the writer to be infested in April 1926, when the plants were about 2 ft. high. Two or three little clusters of the insects occurred on each plant and the grower proposed using calcium cyanide for the control of aphides which were also present. Weekly fumigations, using  $\frac{1}{2}$  oz. per 1000 cu. ft., resulted in

comparative freedom from mealy bug after 8 weeks, and at the end of July the grower stated that he had never had so little trouble with this pest, which this year had caused no damage either to the plants or the crop.

In another glasshouse in Guernsey, weekly fumigations for the control of the mealy bug on vines were commenced, just at the end of the flowering period (May 25th, 1926), with a dosage of  $\frac{1}{4}$  oz. of calcium cyanide per 1000 cu. ft. This dosage was used for several weeks and then increased to  $\frac{3}{4}$  oz. per 1000 cu. ft.

The treatment proved highly satisfactory and the grower stated that, whereas in other years all the bunches of fruit were infested before the end of June, at the end of July 1926 he could not find a single infested bunch.

*Cost of fumigation with calcium cyanide.*

The calcium cyanide method of fumigation compares very favourably as regards costs with the older method of fumigation with sodium cyanide and sulphuric acid. The cost of fumigating a glasshouse of 40,000 cu. ft. at  $\frac{1}{4}$  oz. dosage works out at 1s. 8d. plus the cost of labour, which is small, since the operator has merely to pour the calcium cyanide from the tin into a graduated funnel and read off the required amount. The funnel tap is then turned and the fumigant applied along the paths as the operator walks through the glasshouse.

SUMMARY.

Experiments on glasshouse fumigation with calcium cyanide in Great Britain during 1925-26 indicate that this material is a satisfactory source of hydrocyanic acid gas, and can be used to control a number of the usual pests infesting glasshouse plants. *Trialeurodes vaporariorum* Westw. was held in check with dosages of  $\frac{1}{12}$ – $\frac{1}{2}$  oz. per 1000 cu. ft. and effectively controlled with dosages from  $\frac{1}{4}$ – $\frac{3}{4}$  oz. per 1000 cu. ft. according to the conditions prevailing.

At least six species of greenhouse aphides were controlled with dosages varying from  $\frac{1}{4}$  oz. to  $\frac{1}{2}$  oz. per 1000 cu. ft.

Thrips, of which four species were dealt with, were more difficult to control, apparently only the adults being affected by the hydrocyanic acid gas. Dosages of  $\frac{1}{4}$  to  $1\frac{1}{2}$  ozs. per 1000 cu. ft. killed varying percentages of the adults and a series of fumigations were found to give satisfactory control.

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Two instances are cited where continued fumigations with calcium cyanide apparently resulted in control of mealy bugs on tomatoes and vines in Guernsey.

### REFERENCES.

- (1) AMERICAN CYANAMID COMPANY (1925). *Res. Development of "Cyanogas" Calcium Cyanide*, pp. G. 1-5.
- (2) LLOYD, I. L. (1921). *Sixth Ann. Rept. Cheshunt Res. Stn.* (1920), p. 16.
- (3) MILES, H. W. (1927). *Scottish Journ. of Agriculture*, x, No. 1, p. 63.
- (4) SPEYER, E. R. and OWEN, O. (1926). *Ann. App. Biol.* XIII, No. 1, 144.
- (5) THEOBALD, F. V. (1926). *Plant Lice or Aphididae of Great Britain*, p. 333.
- (6) WALTON, C. L. (1923). *Journ. Min. Agric.* xxx, No. 9.
- (7) WEIGEL, C. A. (1926). *U.S. Dept. Agric., Dept. Circular*, No. 380.

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## THE EFFECT OF SOIL STORAGE AND WATER CONTENT ON THE PROTOZOAN POPULATION

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As it has been conclusively shown that protozoa form a constant constituent of the soil population, and since it is known that their relationship to the other members of that population is an important one, it was decided to re-investigate the effect of certain external conditions on their multiplication and life processes. The present paper deals with the effect of soil storage and of the water content of the soil.

### SOIL STORAGE.

In microbiological studies it has been and still is a common practice to store soils for laboratory experiments in bottles or similar containers for varying periods of time. Within the past few years, however, it has become apparent that such a procedure produces such large changes in both the size and composition of the soil population that accurate experiments are impossible with soils so treated. Lipman<sup>(10)</sup> concludes that in soils kept in pots under greenhouse conditions the filling of the pots and the application of fertilisers involve a more intimate contact of the soil particles with atmospheric oxygen than is possible under field or garden conditions. This leads to an abnormal multiplication of the soil bacteria and to a consequent abnormally rapid oxidation of the organic matter. In the course of time the more readily decomposable portions of the organic matter become depleted and this is followed, in turn, by a decline in the numbers of bacteria. The numbers of the bacteria in his experiments all showed after a period of two months a large decrease.

Waksman<sup>(15)</sup> similarly found that fungi and bacteria in soil kept undisturbed in covered earthenware pots after the first few days showed a general falling off in numbers until the 174th day when the decrease was very slow. Eleven months after the soils had been bottled the numbers of micro-organisms did not change appreciably, a uniform balance of microbiological activity having been reached. Allison<sup>(1)</sup>



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concludes that lack of aeration was a determining factor in causing decrease in numbers during storage of soil.

Fantham and Paterson<sup>(6)</sup> in their work on the South African soils showed that certain species of protozoa persist, though not all survive, in soil bottled for three years; and that the sequence of appearance of the protozoa in cultures is altered after storing; the ciliates from stored soil developing more rapidly in cultures than do those from fresh soil. Goodey<sup>(7)</sup> also obtained an abundant fauna of amoebae and flagellates from soils that had been stored for many years.

It is possible, however, that in all these cases the observers were dealing with the cysts of the protozoa, and that the active forms had disappeared during the period of storage. But since it is imperative, for certain kinds of experiments, that the soil shall be stored and placed under controlled laboratory conditions, it was decided to carry out investigations designed to discover a method by which the soil inhabitants were not injured by such treatment.

The species of protozoa which it was decided to consider were the common soil flagellates *Cercomonas crassicauda*, *Heteromita globosa*, *Oicomonas termo*, and the widely distributed soil amoebae *Dimastigamoeba gruberi*. At first the effect of placing sieved soils in bottles stoppered with cotton wool was tested, since in the past this has been a common method of conducting experiments. The bottles were kept stoppered for weekly intervals when they were opened to permit of taking a sample for counting purposes, and at the same time to adjust the water content which was maintained approximately at one half the water-holding capacity of the soil. Previously to removing the stoppers the soil was violently shaken in order to get uniform distribution, but at no time during the experiment was the soil taken out of the bottles. Counts of the protozoa and bacteria made at weekly intervals for five weeks showed a steady decrease in numbers. This diminution being in respect of the total numbers of the protozoa rather than in respect of the percentage of active forms, which remained fairly high throughout the experiment.

Since this might have been due to the artificial external conditions of the laboratory, a second experiment was carried out similar to the one already described, except that the bottles were buried up to the shoulders in a field, the tops being protected from heavy rain by small tin plates supported above them. As is seen, however, from Table I a decrease in the general population of the bottled soil still occurred even under these somewhat more natural conditions.

Table I.

Days	<i>Cercomonas</i>		<i>H. globosa</i>		<i>Oicomonas</i>		<i>D. gruberi</i>		Bacteria in mil- lions per gram	H <sub>2</sub> O content
	Total	% act.	Total	% act.	Total	% act.	Total	% act.		
At start	172,000	99.1	55,400	98.0	55,400	97.1	18,300	96.5	19.7	21.2
7	9,720	64.4	2,400	0	4,800	63.8	1,200	0	16.4	22.5
14	6,600	0	1,160	0	2,300	0	256	0	13.4	23.8
21	8,500	0	4,700	82.5	2,300	75.2	420	95.6	11.9	23.6
28	6,700	0	1,700	80.3	2,300	27.8	600	0	10.0	24.5
35	4,700	0	2,400	0	1,900	0	1,200	84.2	8.1	23.7

A second experiment made a month later than that given in Table I but otherwise under identical experimental conditions gave similar results.

These two experiments are given as illustrations of many that were made at different periods of the year and with soils which had been variously manured. Since all the experiments gave similar results detailed reference to each is not necessary. There appeared to be two reasons for the effects observed:

(a) the effect of sieving;

(b) that the ratio of the surface area of the soil to its volume was insufficient for adequate aeration.

Experiments speedily eliminated the first possibility for no effect could be observed when sieved soil was compared with unsieved soil. It was decided therefore to concentrate on the second possibility.

Large quantities of soil were sieved and filled into bottles stoppered as before and also into earthenware pots such as are used for pot culture work, the pots having an internal diameter of 10 inches and a depth of 12 inches. The pots and bottles were kept outside and as far as possible protected from rain. The figures already given in Table I were obtained from the bottled soil: those given below in Table II are for the soil in the pots.

Table II.

Days	<i>Cercomonas</i>		<i>H. globosa</i>		<i>Oicomonas</i>		<i>D. gruberi</i>		Bacteria in mil- lions per gram	H <sub>2</sub> O content
	Total	% act.	Total	% act.	Total	% act.	Total	% act.		
At start	172,000	99.1	55,400	98.0	55,400	97.1	18,300	96.5	19.7	21.2
7	8,200	27.4	2,030	50.0	5,720	0	4,500	64.0	18.6	24.2
14	13,200	91.0	1,180	0	6,700	82.4	290	0	21.4	20.5
21	61,000	0	13,400	87.0	13,400	63.0	1,780	91.9	11.3	25.2
28	250,000	97.1	10,300	87.8	3,300	64.5	1,000	82.4	24.3	26.3
35	420,000	95.0	250,000	96.1	350,000	97.5	690	0	16.5	20.4

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Comparison of the two tables shows that the organisms in the pot soil behaved in a much more normal manner than those in the bottled soil. It is true that during the first week of the experiment there was a very marked drop in the numbers of all the species counted, but after this initial depression the population increased and there was an indication of the fluctuations in numbers which are associated with normal field soils. This was also shown by the bacteria. Owing to the pot not being as adequately protected from the weather as were the bottles, the water content of the former varied more during this experiment, but it is not thought that this is sufficient to account for the difference between the two sets of soil.

The final sets of the experiments made were with soil confined in earthenware pots of a shape giving a large surface area. These pots were shallow with a depth of 6 inches, but an internal diameter of 25 inches.

A series of five such experiments were done at different periods of the year, and it was found that after an initial depression, lasting for about three days, the numbers of both protozoa and bacteria remained normal and exhibited the fluctuations already referred to above.

The final series of these experiments were carried out towards the end of 1924 and since that date we have had an opportunity of testing the correctness of our conclusion, viz. that provided the surface area of the soil is large relative to its volume it is possible to carry out laboratory experiments on the micro-organisms of stored soils.

Repeatedly it has been necessary for us to investigate the effect of various treatments on the soil inhabitants; and when a small quantity of soil was needed it has been our practice to use a large Petri dish as the container. This procedure has been found to be quite satisfactory.

### EFFECT OF WATER CONTENT.

Regarding the effect of water content of the soil upon the activities of micro-organisms a certain amount of information has been obtained by early investigators.

Koch (8, 9) and Coppa (2) consider moisture to be the most important factor in the growth of soil protozoa. Koch's results indicate that the largest number of protozoa were recorded when the moisture was highest ( $1\frac{3}{4}$  of optimum) and at the lowest ( $\frac{1}{3}$  of optimum) protozoa did not become active. Waksman (14), on the contrary, states that "the soils with the optimum moisture gave in the main higher bacterial numbers . . . than those containing full moisture holding capacity."

Cunningham (3) supports Koch's view, since he finds that the number

of protozoa is increased in the soil with a moisture content of 70 per cent. water-holding capacity and in saturated samples, where flagellates develop to the exclusion of amoebae. Waksman (13) finds that flagellates are present in soil with a moisture content of 14 per cent. which is too low for the development of the other groups. Sherman (12) also states that small flagellates will develop in soil of 11 per cent. water-holding capacity.

As the conclusions just cited were obtained from experiments in which either the direct method of counting was used or else where no distinction had been drawn between active and cystic stages of protozoa, it seemed desirable to re-investigate the problem using the methods elaborated in this laboratory (4); and counting the numbers of active and cystic forms of the same species of protozoa as were used for the storage experiments.

In the first set the soil was air dried and then moistened until it attained a water content equal to  $\frac{1}{3}$  of the water-holding capacity. Owing to the drying all active protozoa were killed, but the animals in the cystic condition remained viable. Counts made at varying periods after the soil had been moistened would show whether there was sufficient water to allow of excystation and subsequent reproduction.

Two counts were made at intervals of  $1\frac{1}{2}$  hours and 24 hours after the soil had been wetted. In only one case, that of *Cercomonas crassicauda*, did excystation and reproduction occur, the numbers of this animal increasing from 64,000 per gram to 230,000 in 24 hours.

The numbers of bacteria, which at the beginning of the experiment were 6.6 millions per gram, also showed an increase to 11.69 during the same period. Repetition of this experiment gave similar results.

It was therefore decided to carry out similar experiments with increasing water contents and at the same time to enumerate the numbers of active protozoa in addition to the total numbers. The results are given in Tables III, IV.

From these two experiments it would appear that soil having a water content of one-third or above its water-holding capacity provides a suitable medium for the excystation and reproduction of these four common soil protozoa and that at  $\frac{1}{3}$  of the water-holding capacity only the species *Cercomonas crassicauda* is capable of survival.

It will also be noticed from the figures given in the tables that the organisms exhibited the same kind of fluctuations both in total numbers and the proportion of active and cystic forms as has been shown in earlier papers (4) to be characteristic of normal field soils.

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Table III.

	Soil air dried and water content brought to $\frac{1}{2}$ w.h.c.									
	At start		After 1½ hrs		After 4½ hrs		After 24 hrs		After 48 hrs	
	No.	% act.	No.	% act.	No.	% act.	No.	% act.	No.	% act.
<i>Cercomonas</i>	230,000	0	95,000	88.4	64,000	43.7	230,000	85.6	140,000	83.7
<i>H. globosa</i>	21,000	0	21,000	85.6	3,600	50.0	44,000	95.9	5,100	67.5
<i>Oicomonas</i>	10,000	0	5,100	64.9	5,100	87.4	10,000	87.0	5,100	40.9
<i>D. gruberi</i>	1,800	0	1,800	76.2	2,800	0	1,800	65.2	900	84.6

Table IV.

	Soil air dried and water content brought to $\frac{1}{2}$ w.h.c.									
	At start		After 3 hrs		After 6 hrs		After 24 hrs		After 72 hrs	
	No.	% act.	No.	% act.	No.	% act.	No.	% act.	No.	% act.
<i>Cercomonas</i>	44,000	0	95,000	37.8	230,000	84.3	420,000	85.9	420,000	85.9
<i>H. globosa</i>	41,000	0	44,000	0	15,000	80.2	140,000	83.6	140,000	96.2
<i>Oicomonas</i>	1,800	0	1,800	40.2	10,000	66.8	44,000	91.6	21,000	82.4
<i>D. gruberi</i>	7,300	0	10,000	20.1	15,000	26.6	21,000	87.6	30,000	87.6

In order to test how far these laboratory results are a true picture of what takes place in a field under normal environmental conditions, advantage was taken of a dry spell of weather in 1924 under which certain of the Rothamsted soils became deficient in water. Samples from the top four inches of the surface soil of one of the Barnfield plots were taken daily for three days and gave water contents of 6.4 per cent., 6.2 per cent. and 6.1 per cent. respectively, the water-holding capacity of the soil being 37.9 per cent. The results of the counts are given in Table V.

Table V.

	1st day		2nd day		3rd day	
	Total no.	% active	Total no.	% active	Total no.	% active
<i>Cercomonas</i>	64,000	94.2	15,000	67.5	44,000	87.9
<i>H. globosa</i>	2,600	0	2,600	0	2,600	0
<i>Oicomonas</i>	1,300	0	640	0	640	0
<i>D. gruberi</i>	1,800	0	900	0	1,300	0

These results are therefore in agreement with those obtained from laboratory experiments, and it is of interest to note that again the flagellate *Cercomonas* is capable of living in an active condition under conditions of extreme dryness.

Finally in another experiment soil was taken at various depths from two plots of Hoos Field: one of the plots was fallow, the other being cropped with wheat—Table VI.

Table VI.

Depth	Fallow						Cropped					
	0-2 in.		2-4 in.		5-6½ in.		0-2 in.		2-4 in.		5-6½ in.	
	Total	% act.	Total	% act.	Total	% act.	Total	% act.	Total	% act.	Total	% act.
<i>Cercomonas</i>	160	68.7	230	56.1	320	50.0	110	73.0	450	61.0	320	24.7
<i>H. globosa</i>	25	0	25	0	160	0	38	0	56	0	56	0
<i>Oicomonas</i>	25	0	79	0	25	0	25	0	25	0	0	0
<i>D. gruberi</i>	160	0	110	0	110	0	110	0	230	0	160	0
Water content	4.25		4.3		4.5		6.6		6.7		6.8	

Here again it is found that except in the case of *Cercomonas* activity was not possible for the protozoa at such low water content. -

It would seem therefore that low water content of the soil constitutes a limiting factor for the development of protozoa, but that once the value rises above the limiting one,  $\frac{1}{8}$  to  $\frac{1}{2}$  the water-holding capacity, further increase in the amount of water does not have a very marked effect.

This view finds support in the results obtained by Cutler, Crump and Sandon(4) in 1922. The numbers of bacteria and of six species of protozoa were counted daily for a year. Throughout this period, except for the last nine days, the water content of the soil varied between 12 per cent. and 22 per cent., but no correlation could be found between the numbers of micro-organisms and the amount of soil water. During the last nine days of the experiment the soil gradually dried and there was an indication that this was resulting in a diminution in the number of active forms of the protozoa, and a consequent increase in the size of the bacterial population.

Sandon(11) in his work on the *Distribution of Protozoan Fauna of the Soil* discussing this question writes "that the statement frequently made...that moisture is the principal controlling factor, though very plausible, appears to be equally unfounded."

Provided that the moisture contents in question were above the limiting value, as was the case in the soils discussed by Sandon, this view would appear to be perfectly correct.

#### SUMMARY.

1. Experiments are described showing that it is possible to conduct laboratory experiments on micro-organisms in stored soil, providing the surface of the soil is large relatively to its volume.

2. Experiments on the influence of moisture on the soil protozoa

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show that active forms are found in soils containing a very low percentage of water.

3. The flagellate *Cercomonas crassicauda* was found to excyst and reproduce in air-dried soil brought to  $\frac{1}{2}$  the water-holding capacity. The numbers of the bacteria also in this experiment nearly doubled their numbers in 24 hours.

4. Four common soil protozoa were found to behave normally in soil of  $\frac{1}{2}$  and  $\frac{1}{3}$  the water-holding capacity, which previously had been air-dried and then brought to the desired water content.

5. Field soils having water contents of 6 per cent. in one case and 4 per cent. in another corroborated the laboratory experiments. In all of these samples active *Cercomonas* were found, other protozoa being present only in the form of cysts.

### REFERENCES.

- (1) ALLISON, F. E. (1917). Biological changes in soils during storage. *Soil Sci.* III, 37-62.
- (2) COPPA, A. (1921). Researches on protozoa of soils and water. *Exp. Sta. Record*, XLVIII, 817.
- (3) CUNNINGHAM, A. (1915). Studies on soil protozoa. *Journ. Agric. Sci.* VII, 49-74.
- (4) CUTLER, D. W., CRUMP, L. M. and SANDON, H. (1922). A quantitative investigation of the bacterial and protozoan population of the soil, with an account of the protozoan fauna. *Phil. Trans. Roy. Soc. London*, B, 211, pp. 317-50.
- (5) FANTHAM, H. B. and PATERSON, N. F. (1923). Some protozoa in certain South African Soils. III. *South African Journ. Sci.* XX, 437-92.
- (6) ——— (1924). Some protozoa in certain South African Soils. IV. *Ibid.* XXI, 445-79.
- (7) GOODEY, T. (1915). Investigations on protozoa in relation to the factor eliminating bacterial activity in soil. *Roy. Soc. B*, LXXXVIII, 437-56.
- (8) KOCH, G. P. (1915-16). Activity of soil protozoa. *Journ. Agric. Res.* V, No. 1, 477-88.
- (9) ——— (1916). Studies on the activity of soil protozoa. *Soil Sci.* II, 163-81.
- (10) LIPMAN, J. G. and OWEN, I. L. (1910). Some bacteriological relations in soils kept under green-house conditions. *Journ. Agric. Sci.* III, 301-10.
- (11) SANDON, H. (1927). The composition and distribution of the protozoan fauna of the soil. *Biological Monographs and Manuals*. Oliver and Boyd, Edinburgh.
- (12) SHERMAN, J. M. (1916). Studies on soil protozoa and their relation to the bacterial flora. *Journ. Bact.* I, 35-66, 165-85.
- (13) WAKSMAN, S. A. (1916). Studies on soil protozoa. *Soil Sci.* I, 135-52.
- (14) ——— (1916). Protozoa as effecting bacterial activities in the soil. *Ibid.* II, 363-76.
- (15) WAKSMAN, S. A. and STARKEY, R. L. (1923). Partial sterilisation of soil, microbiological activities and soil fertility. II. *Ibid.* XVI, 247-68.

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## REVIEWS

*The Insects of Australia and New Zealand.* By R. J. TILLYARD. Pp. xi + 560, with 44 Plates and 468 Text-figures. Sydney: Angus and Robertson, 1926.

Dr Tillyard is one of the most prolific and original entomological investigators of the present generation and it is seldom that he is unable to shed some fresh light or new interpretation on whatever problem he may be engaged. Those who look for originality in the present textbook will have little cause for complaint and, although intended primarily for students in Australia and New Zealand, it contains much that will appeal to a wider circle. The author's intimate knowledge of the insects of those two lands renders him well qualified for his task, and his wide knowledge of underlying principles has enabled him to produce a volume far above the level of a faunistic handbook.

The book has been wholly set up and printed in Australia and the result is excellent. The type is clear and there is a wealth of mostly original illustrations. Of the 1251 separate figures, 203 are coloured and are reproduced on plates. On the whole these coloured illustrations serve their purpose admirably and enable the species thus portrayed to be easily recognised. Great credit is due to Mrs Tillyard's skill in drawing the originals of so many of the plates. Another pleasing feature of this volume is the small number of misprints or other typographical errors.

The arrangement of the subject-matter is in the form of chapters; six of the latter are of the nature of generalities, while the remaining 24 chapters are each devoted to a separate order of insects. In the space at command it is only possible to comment here and there on the most important sections of the book. The general classification given in Chap. I offers little scope for criticism. Dr Tillyard divides the Insecta into 24 orders and wisely rejects the modern pluralistic tendencies which recognise as separate orders groups of no higher than sub-ordinal rank. The Anoplura are held to include the Mallophaga to which they are closely related: the author, however, has some doubts respecting the ordinal value of the Zoraptera which might quite well have been merged as a division of his Copeognatha. The evidence for the Zoraptera being social, rather than simply gregarious in habit, rests on very slender evidence. Chap. II deals with external morphology and can be recommended as one of the best introductions to the subject that is available. Exception, however, must be taken to the author designating the dorsal and ventral plates of the abdominal segments as tergites and sternites, rather than using the established terms terga and sterna respectively. On p. 10, with reference to segmentation, Dr Tillyard correctly defines the tergites as the separate sclerites of a tergum and the sternites as the sclerites of a sternum. Chap. III dealing with internal anatomy is naturally brief in a work of this description: the author is evidently not at his best in discussing this subject and not entirely accurate as regards matters of physiology. The subject of Life History forms Chap. IV and includes the essential facts of metamorphosis in so far as external changes are concerned. The immature forms of all metabolous insects are termed larvae, the author objecting to the word nymph, in the case of the Hemimetabola, as being unscientific and there is a good deal to be said in favour of his view. Chaps. V, VI and VII are concerned with the Thysanura, Protura and Collembola respectively. With reference to the Thysanura, an error has crept in regarding the spiracles. The author gives "7 or 8 pairs" for this order whereas there are 9 pairs in the Machilidae, 10 pairs in *Projapyx* and the Lepismidae, and 11 pairs in certain species of *Japyx*. In the Campodeidae, Dr Tillyard states that spiracles are absent, but as a matter of fact, there are three pairs in *Campodea*. It is difficult to agree with his



reasoning with regard to the styli associated with the two hind pairs of legs being homologous with epipodites. The latter structures in Crustacea are borne on the coxopodite (sub-coxa in Machilidae) whereas in *Machilis* they are carried on the basipodite or coxa which suggests that they are the homologues of exopodites. Chap. VIII is devoted to the Plecoptera or May-flies and in this account the author has incorporated his recent researches on the venation which have shed fresh light upon its interpretation. The Odonata (Chap. IX) are discussed in quite a masterly fashion and the intricacies of their morphology very clearly explained. Chap. X provides an excellent guide to the Orthoptera which are abundantly represented in Australia. The Isoptera or Termites follow and with regard to these insects it is unusual to find an up-to-date writer adhering to the idea of the existence of neoteinic royal forms. Recent work demonstrates the probability that such castes are true adults in a brachypterous or an apterous state as the case may be. The Perlaria or Stone-flies (Chap. XIII) form a short section, but Dr Tillyard has made good use of the primitive forms of the Southern Hemisphere in what he has to say respecting these insects. The Copognatha or Psocoptera (Chap. XVI) have come in for overhauling and are regrouped on more rational lines in the light of the author's own discoveries among the Lower Permian fossils. Passing on to Chap. XIX, the Hemiptera, there will be found an excellent account of an order of great economic importance. The stumbling-block of a satisfactory classification of the Fulgoroidea has been overcome with help from Mr F. Muir, and this feature is a valuable aid to the identification of the numerous species. The long chapter on Coleoptera is well justified since there are over 21,000 species of the order in the two lands concerned. The family Curculionidae heads the list in sheer number of forms and some very remarkable representatives are figured. Mention needs also to be made of the curious Longicorn *Tillyardua* and the interesting figure showing its remarkable pectinate palpi. Chap. XXII, on Hymenoptera, is unique in that the author has done good service in clearing up the greatly involved vein-nomenclature that has so long been a drawback. By applying the results of his studies on *Protolymen* and other types he has been able to simplify the Comstock-Needham system as applied to these insects and reduce its apparent anomalies into an orderly system. The rich Neuropterous fauna of Australia is well indicated in the chapter concerned and a number of little known larval types are figured. In treating of the Diptera, that group is subdivided into two main sections—the Nematocera and Brachycera. This somewhat heterodox arrangement is a welcome step over the more usual system and is in conformity with what was probably the primitive dichotomy of the order. The account of the ptilinum is somewhat obscure, and on p. 334 this organ is mentioned as lying within a cuticular "infolding" known as the frontal lunule. On this explanation it is difficult to imagine how so large an organ as the ptilinum can be evaginated from beneath what is apparently a hard sclerite, since no mention is made of the extensive  $\Omega$ -shaped frontal suture through which the ptilinum is usually regarded as being expelled. On p. 365 it is stated that the frontal lunule and ptilinum are absent in the Syrphoidea. This is scarcely correct since the lunule is clearly demarcated in the Syrphidae and in some cases at least a functional ptilinum occurs, even though it may not persist. It appears from these remarks that the author may have confused the frontal lunule with the frontal suture which is wanting in the Syrphoidea or at most but meagrely developed. The chapter on Lepidoptera is perhaps one of the best ever written in a general textbook. The classification presented is a new one based on the work of Turner and is evidently designed to overcome the outstanding difficulty of grouping the large number of families into rational major divisions. The super-families adopted bring together, in many cases, in natural association closely related families and in this respect marks an advance on existing systems. The volume concludes with an excellent chapter on the fossil insects of Australia and New Zealand and a useful section on entomological technique. There is also a glossary of technical terms used and a very complete index of subjects and authors. The bibliographies are supplied at the end of each chapter concerned and include the essential literature, especially works of a taxonomic character.

A scholarly book of this character will prove a great stimulus to entomology in

the Antipodes. It is no small problem to survey a fauna comprising over 40,000 species, note practically all the important genera and diagnose over 400 separate families. Dr Tillyard has carried out his task with conspicuous success and the few errors here noted fade into insignificance in face of the essential merits of the whole work.

A. D. IMMS

*The Aspergilli.* By CHARLES THOM and MARGARET B. CHURCH. Baillière, Tindall and Cox, 1926. 22s. 6d.

The volume is divided into two portions, a general discussion and a detailed taxonomic study. Part one commences with a history of the genus, its morphology, and a discussion of the basis of description and classification. Culture methods are described and this leads to chapters on the physiology and biochemistry of the genus, more particularly enzymic and fermentative activities in relation to industry, and finally to *Aspergilli* in relation to animal disease. Part two deals with the nomenclature of the genus and its division into groups and contains full descriptions of individual species and doubtfully related forms. The volume concludes with a synoptical key to the *Aspergilli*, a list of sixty-six accepted species, eight and a half pages of references and an index. There are also fourteen text-figures and four plates of micro-photographs.

The book is somewhat uneven in treatment. The discussion of morphology and cytology might perhaps be improved, whilst on the other hand the chapters on physiology are quite masterly summaries. The value of the detailed taxonomic portion can only be judged by its use in practice, but the keys have been tried out on a number of soil *Aspergilli* in culture and found extremely convenient and critical. The bibliography is not arranged alphabetically but according to textual reference which somewhat detracts from its convenience. Also it is not so complete or up-to-date as might be desired. Apart from the use of the more unusual form of certain words such as "somber," "mollusk," "vigor" and "color" the language is extremely clear and unambiguous. It is a pity that the binding of the volume is not stronger—of four copies that I have seen, pages are already loose in three, and this is a serious matter in a book that is essentially for constant use in the laboratory.

Any small criticisms, however, sink into insignificance beside the thanks that all micro-biologists must give to the authors for producing this monograph. No more difficult task could have been attempted, and that Dr Thom and Miss Church had the courage to assay it and carry it through to so successful an issue puts all students of the culture of micro-organisms in their debt.

WILLIAM B. BRIERLEY

*Potato Varieties.* By R. N. SALAMAN. Cambridge University Press, 1926. 25s.

This book is one of the most refreshingly original volumes that have appeared for some time. It is an intensely personal book, being in no sense an impartial and complete compilation of other people's views on the subject but the writing down by Dr Salaman of Dr Salaman's views, the results of Dr Salaman's investigations and the accumulated wisdom and experience of Dr Salaman's two decades of interest and research on the problems. There are very obvious lacunae in the book—indeed, even as a treatise on potato varieties, it is quite incomplete—and one can only feel glad that the author has resisted the temptation to fill in these gaps with second-hand knowledge for the sake of completion. Sooner or later they will come within the scope of Dr Salaman's personal work, for he has amazing energy and is almost omnivorous, and one looks forward to successive editions in which his personal observations on these issues also will be given. As it is the book is packed full of first-hand observations, acute suggestions and facts impossible to obtain elsewhere, all

dealing with topics selected by their appeal to Dr Salaman's interests, but of primary importance to all dealing with agricultural plants.

The wide-ranging character of the work will be appreciated by the chapter headings which are as follows: (I) The definition of a potato variety; (II) Historical sketch of the development of present-day varieties; (III) The life of a variety; (IV) The methods of producing new varieties; (V) The application of genetics to variety raising; (VI) The technique of hybridization; (VII) The classification of varieties; (VIII) Classificatory guide; (IX) Correlations or linkages; (X) Varietal differences of maturity; (XI) The yield or crop of the potato, and the genetic factors affecting the same; (XII) The yield or crop of the potato and the environmental factors affecting the same; (XIII) Yield trials; (XIV) Varietal differences in the production of ware; (XV) Varietal differences and their basis in chemical and histological characters of the tuber; (XVI) Differential histological features of the tuber; (XVII) Varietal resistance of disease; (XVIII) Virus diseases and varietal resistance to their infection; (XIX) Degenerative diseases of unknown origin; (XX) Varietal differences in respect to extremes of temperature; (XXI) The adaptation of varieties to special conditions of soil and climate; (XXII) Synonymy; (XXIII) The leaf index; (XXIV) Varietal descriptions; (XXV) Common employment of the varieties described; (XXVI) Descriptions of varieties with their history and synonyms. In addition, there are three appendices, one by Mr W. H. Parker on Yield Testing, a Bibliography, an Index and a detailed Contents. The mere citation of the contents headings shows the range of the book. Many of the chapters, however, are very short, two or three pages only, but others dealing with problems, where the author is more at home, are fuller, that on variety description for example, running to over 100 pages.

Hardly anyone, least of all the author, could carry out active experimental research on a congeries of such important problems for nearly two decades without accumulating a vast amount of valuable general experience. Very often such general experience is of a kind that cannot be included in formal research papers, especially in these days of compression, and usually therefore is lost to all other workers on the problems. In the present volume, however, the author has created his opportunity and produced a book which is at once notable largely because it is charged so richly with this general experience.

The vigorous personality of Dr Salaman runs through every page, at times in a most amusing manner. Thus his very first sentence rides roughshod over all philosophical adumbrations on problems of individuality and race, and, of one of the most contentious examples, asserts bluntly "no matter how it may have been produced, the fact must never be overlooked that a variety is in reality an individual and not a race." This intensely personal standpoint is maintained throughout and adds greatly to the enjoyment of reading the volume. The book is beautifully produced and illustrated by ten plates, of which three are in colour, and is a volume that should be readily available to all students and research workers in agriculture and botany.

WILLIAM B. BRIERLEY

## ASSOCIATION OF ECONOMIC BIOLOGISTS

### REPORT OF THE COUNCIL FOR THE YEAR 1926, PRESENTED TO THE ANNUAL MEETING, JANUARY 28TH, 1927

No important change in the style of the proceedings was made last year; six meetings devoted to papers and discussions were held at the Imperial College of Science to whose authorities the Council again desires to express its sincere thanks for the facilities afforded to the Association. These meetings were attended not quite so fully as might have been expected considering the number of members who live within easy distance of London, and the Council hope that greater enthusiasm may be shown in the ensuing years.

The Annual Provincial Meeting was to have been held at the Long Ashton Horticultural Institution but the industrial upheaval came along just when all the preparations for the meeting had been made, and the difficulty of railway transport was so great that it was decided to cancel the meeting. The Council desire to express their thanks to the Vice-Chancellor of Bristol University and to Prof. B. T. P. Barker and his colleagues for the trouble they had taken in preparing for the meeting and to record their hope that the invitation to visit this Institute may be renewed at some early date.

The Summer Meeting was held at the Research Station at Cheshunt and thanks to the excellent organisation of the Director, Dr W. F. Bewley, a very profitable and enjoyable day was spent there.

The Council grievously laments the loss it has sustained in the death of one of its members, Prof. F. W. Gamble, whose place has been taken by Dr J. W. Munro. During the year the Association has lost four members by death, ten members have resigned and ten new members have been added to the Roll which now stands at a total membership of 229.

The Council desires to make known that it has decided that steps shall be taken in the near future to register the Association under Section 20 of the Company's Consolidation Act of 1908, a step which in the opinion of the Council will be of benefit to the Association.

Papers read to the Association during the year 1926.

- Jan. 29.* Prof. V. H. BLACKMAN, Presidential Address: "Recent Work in Plant Pathology and its relation to Applied Botany."
- Feb. 26.* H. V. TAYLOR: "The Importance of Applied Botany to Modern Fruit Growing."
- Mar. 26.* LORD LOVAT: "Forestry in Relation to Science—The National Economic Aspects."  
Professor A. W. BORTHWICK: "Forestry in Relation to Science—The Points of Contact of Forestry and Economic Biology."
- Oct. 22.* R. G. HATTON: "The Influence of the Root Stock on the Vigour, Productivity and General Behaviour of the Apple Tree."
- Nov. 12.* C. L. WITHERCOMBE: "The Sugar-Cane Frog Hopper Problem in Trinidad, a study of its Factors and Principles of Control."
- Dec. 10.* A. J. RIKER: "Studies of Crown Gall and Wound-overgrowths on Apple Nursery Stocks."  
H. WORMALD: "Crown Gall with reference to its occurrence on Fruit Stocks in this Country."

REPORT OF THE HON. TREASURER FOR THE  
YEAR 1926, PRESENTED TO THE ANNUAL MEETING,  
JANUARY 28TH, 1927

The annual statement of the accounts of the Association for the year ending December 31st, 1926, is set out on p. 261. It has been our custom in the past to meet the expenses of the previous year's volume of the *Annals of Applied Biology* out of the revenue of the current year. This has been inevitable owing to the lateness in the appearance of the last part of the current volume for a number of years in succession. For the last two volumes this disadvantage has been largely eliminated and it is hoped in the near future to pay the expenses incurred by each volume out of the revenue of its own year. In order to make this adjustment it will be necessary to pay for two volumes out of a single year's income for one year only, but once this is accomplished the recurrence of the difficulty is not anticipated.

During the year 1926 current subscriptions amounted to £252. 14s. 2d. as compared with £220. 9s. 0d. for the previous year. Special efforts have been made to urge prompt payment of the annual contribution to the Association and the response thereto has left little cause for complaint. At the same time a great advance has been made in the payments of arrears, £54 has been received under this category as compared with only £7. 10s. 0d. for 1925.

The working expenses of the chief charge on the Association, viz. the *Annals of Applied Biology*, have been considerably lower for vol. XII than for the volume for the previous year. This reduction is entirely due to an increase in revenue derived from the sale of that volume and of back volumes and parts. The amount required to meet the publishers' charges for vol. XII is £126. 11s. 9d. as compared with £303. 1s. 0d. for vol. XI. Allowing for the cost of vol. XIII which is to be met out of our 1927 revenue, our assets exceed our liabilities by £622. 3s. 9d., of which £574. 2s. 5d. are represented by cash as is shown in the balance sheet.

A. D. IMMS,  
Hon. Treasurer.

## TREASURER'S STATEMENT FOR THE YEAR ENDING DECEMBER 31st, 1926

### CASH ACCOUNT.

<i>Cr</i>	£	s.	d.	<i>Dr</i>	£	s.	d.
Jan. 1. Cash at Bank . .	70	8	6	Postage . . . . .	6	5	6
Dec. 31. Subscriptions:				Stationery and minor printing	8	16	11
A. Current . . . .	252	14	2	Treasurer . . . . .	136	17	8
B. Arrears . . . .	54	6	0	Secretaries . . . . .	6	19	6
C. Advances . . . .	12	10	0	Balance at Bank . . . .	71	13	3
Entrance Fees . . . .	4	4	0	Placed on Deposit . . . .	200	0	0
Contributions to cost of papers in <i>Annals</i> . .	28	12	6				
Bank Interest . . . .	7	17	8				
Total	£430	12	10	Total	£430	12	10

### BALANCE SHEET.

LIABILITIES.	£	s.	d.	ASSETS.	£	s.	d.
Subscriptions in advance . .	12	10	0	Current a/c . . . . .	71	13	3
Liability on <i>Annals</i> , vol. XIII	408	15	8	Deposit a/c . . . . .	430	0	0
Excess of assets over liabilities	622	3	9	Subscriptions two years or less in arrears and considered good	5	0	0
				National Savings Certificates .	481	5	0
				Estimated value of stock of <i>Annals of Applied Biology</i> with publishers . . . . .	55	11	2
Total	£1043	9	5	Total	£1043	9	5

A. D. IMMS, *Treasurer*.

We have examined the Treasurer's statement of expenditure and receipts and have found it correct. We consider that the above balance sheet correctly represents the position of the Association.

C. T. GIMMINGHAM.  
GEO. H. PETHYBRIDGE.



ON FORMS OF THE HOP RESISTANT TO MILDEW  
(*SPHAEROTHECA HUMULI* (DC.) BURR.);  
VI. LOSS TEMPORARY OF IMMUNITY

BY PROF. E. S. SALMON.

(*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*)

DURING 1926 a case was observed where three varieties of hops (*Humulus Lupulus*) which had previously shown complete resistance to the attacks of the "powdery mildew" *Sphaerotheca Humuli* lost, temporarily, this immunity. The details concerning each variety will first be given separately and then the factors possibly concerned with the loss of immunity will be considered.

VARIETY 1. THE GOLDEN HOP.

*Origin.* The exact origin of the Golden Hop—a horticultural variety used for decorative purposes—is unknown, but it appears to have originated on the Continent ((1), p. 293).

*Evidence as to Immunity.* Plants of this variety, grown in pots in an unheated greenhouse, were first tested in 1916 and were found to be completely resistant to the attacks of the mildew. Details of the experiments have already been given ((2), p. 88). Further inoculation experiments to test its immunity were carried out in 1917, and some of these, as was then stated ((2), p. 89), took place "under abnormal weather conditions, when rapid changes of temperature occurred." No infections resulted and the conclusion was reached that "the abnormal weather conditions had no effect upon the immunity" of this variety. It was again tested in 1918 and 1919; 47 plants in pots were repeatedly inoculated throughout the growing season and remained persistently immune ((1), p. 296).

*Partial break-down of Immunity in 1926.* In the spring of 1926, the mildew was found for the first time on the Golden Hop. The material affected consisted of (a) old and (b) young plants, and the details observed were as follows.

(a) By April 28th, a plant of the Golden Hop which has been grown in the greenhouse for a number of years, had produced a shoot 8.5 cm.



long, with six pairs of leaves. At this date one leaf at the 2nd node from the tip bore two minute "powdery" patches<sup>1</sup> of the conidial stage of *S. Humuli*, both situated on the extreme margin of the terminal lobe (one on each side) and near its base. On May 10th, by which date the shoot had not elongated, owing to the weather conditions, the two minute powdery patches were persisting but had not appreciably increased in size; a third minute, densely powdery patch had appeared on the lamina of the same leaf, at a place opposite and close to one of the sinuses at the base of the terminal lobe. Further, a very minute powdery patch was present on a leaf at the next node above.

All the above-described patches remained powdery for some weeks without noticeably increasing in size, and then slowly died away. No further infection of this plant occurred. During May and June the shoot grew to several feet in length, and other shorter shoots appeared. Although the plant was kept in the greenhouse throughout the season, exposed continually to infection by conidia blown on to it from a large number of adjacent mildew-infected hop plants, all the leaves remained healthy, just as they had invariably done in previous seasons.

Table I.

Date of exam.	Pot	Length of shoot (cm.)	No. of pairs of leaves	Condition
Apr. 28	1	15.5	6	All healthy
May 11	1	18.5	8	Two leaves, both at the 4th node from tip, with three minute powdery patches, the largest measuring 1 × 1 mm. All the patches were near the edge of the leaf
Apr. 28	2	14.4	6	All healthy
May 11	2	30.0	9	All healthy. One new shoot, 6 cm., four pairs leaves, all healthy*
Apr. 28	3(a)	6.5	3	All healthy
May 11	3(a)	11.5	5	"
Apr. 28	3(b)	2.5	1	"
May 11	3(b)	5.5	3	"
Apr. 28	4	13.5	6	"
May 11	4	18.0	8	One very minute powdery patch at margin of one leaf at the 4th node from tip†
Apr. 28	5	9.0	5	All healthy
May 11	5	10.0	6	One minute powdery patch on a leaf at 2nd node from tip

\* This plant was used in Exp. 5. (See p. 271.)

† This plant was used in Exp. 1. (See p. 265.)

<sup>1</sup> The term "powdery" patch is used to denote the appearance given by densely clustered conidiophores which have produced a powder-like mass of conidia.

(b) The material consisted of five pots of cuttings of the Golden Hop taken in the previous autumn from a plant (Ref. No. 288) growing in the Experimental Hop Garden at Wye College. Examination of the plants on April 28th and on May 11th gave the results shown in Table I.

The powdery patches did not increase appreciably in size, and died away in the course of a few weeks. All the plants grew normally and produced by early summer vigorous stems several feet in length; the leaves of these, although exposed repeatedly to inoculation in the greenhouse, showed the complete immunity which had been observed in previous seasons.

*Inoculation Experiments with the Golden Hop.* In three experiments, leaves were inoculated<sup>1</sup> with conidia from various sources.

*Exp. 1.* May 5th. One of the plants (pot 4) noted above, was used; two leaves (just expanded) at the 3rd node from the tip were inoculated with conidia, each at three marked places: one leaf, *a*, from the mildew growing on a very susceptible seedling hop (Ref. No. B 18) and the other, *b*, from the mildew on another susceptible seedling of unknown origin. On May 13th leaf *a* showed a very minute cluster of conidiophores at one place of inoculation. No further growth of the fungus took place on this leaf. On May 17th leaf *b* showed at one of the marked places a very minute powdery patch and also, near by, a cluster of five conidiophores. On May 20th another very minute powdery patch had appeared near the margin of the leaf. By May 31st the older patch had died away. By June 16th all the mildew patches had died away on the inoculated leaves, and the 22 leaves produced by this date above the inoculated leaves were all healthy. The plant was then placed in a greenhouse among plants covered with mildew where it was exposed continually to infection; it remained, however, persistently immune.

*Exp. 2.* May 12th. One of the plants (pot 3) noted above was used; one young leaf at the 2nd node from the tip was inoculated with conidia of the mildew growing on the seedling hop of unknown origin referred to above. Conidia from the same source were also placed at the same time on a similarly situated leaf of the very susceptible seedling B 18. The "control" leaf at the same node, of each plant, was moistened with water. Nine days after inoculation, the leaf of B 18 was fully infected, while no infection occurred on the Golden Hop. The same results were visible on the 21st day. The two "control" leaves remained free from mildew.

*Exp. 3.* May 17th. One of the plants (pot 2) noted above was used. One leaf at the 3rd and 4th nodes were inoculated with conidia from the seedling hop of unknown origin. Conidia from the same source were also placed at the same time on two similarly situated leaves<sup>2</sup> of a cutting of a "wild" hop which was growing in a pot. The four opposite "control" leaves were treated with water. By May 25th the leaves of the "wild hop" were fully infected, being covered with mycelial growth

<sup>1</sup> The method of inoculation consisted in moistening the surface of the leaf with distilled water and placing conidia thereon. In a short time the water evaporated and left the conidia deposited on the leaf.

<sup>2</sup> Both the inoculated leaves at the 3rd node were only partly expanded.

bearing densely clustered conidiophores; the control leaves were free from mildew. No infection resulted on the inoculated leaves of the Golden Hop. Very surprisingly, however, on May 31st a few minute clusters of conidiophores, the result of infection from some unknown source, were apparent at the edge of one leaf at the 5th node, and also towards the middle of the control leaf at the 4th node. By June 16th all the patches had died away. No infection took place otherwise on the 16 leaves produced above the inoculated leaves.

In a series of experiments, the details of which are given in a paper published in the present number of the *Annals* (see below, p. 280) scions of the Golden Hop, grafted on a "wild hop" stock, were inoculated with conidia. In two experiments very slight infection resulted, with the production of a few conidiophores; in six the leaves proved to be immune. The dates of the inoculations in the first two experiments were April 29th and 30th; of the cases where negative results were obtained, May 5th, 10th, 19th, 25th. The grafted shoots of the Golden Hop grew vigorously and by early summer had reached a length of several feet; the plants, after resisting inoculation by hand, were placed in a greenhouse and exposed to continual infection for several weeks, and all remained persistently immune.

It is clear from the facts given above that there was no general breaking down of the immunity of the Golden Hop throughout the growing season in 1926, but only a manifestation of a slight and temporary susceptibility at a certain period.

#### VARIETY 2. THE SEEDLING HOP, Ref. No. X 91.

*Origin.* This seedling was raised at Wye College in 1917, the female parent being the Golden Hop and the other an unknown English male hop.

*Evidence as to Immunity.* Two cuttings, taken in 1919, were grown in pots in 1920 in a greenhouse and remained immune although exposed to continual infection throughout the growing season.

*Partial breakdown of Immunity in 1926.* The material on which observations were made consisted of 13 cuttings in pots, taken in 1925 from three clone plants (Ref. Nos. OG 9, OH 3, OI 34) derived from the original plant of X 91 in the Experimental Hop Garden at Wye. These plants, stationed in a cold greenhouse, were examined on April 28th and May 11th with the results shown in Table II.

The powdery patches did not increase appreciably in size after May 11th, and very gradually died away. The conidia produced did not apparently cause further infections of the plants. During May and June the shoots of all the plants grew to several feet in length and although

Table II.

Date of exam.	Plant	Length of shoot (cm.)	No. of pairs of leaves	Condition
Apr. 28	OG 9 (1)	3.5	4	One leaf with two minute powdery patches
May 11		8.5	6	Five leaves with small powdery patches, two densely powdery, $4 \times 2$ and $2 \times 2$ mm.
Apr. 28	" (2)	1.0	3	Healthy
May 11		8.5	3	One leaf with powdery patch; lateral shoot with two pairs of leaves, young mildew patch on two leaves
Apr. 28	" (3)	4.5	5	Three leaves with minute powdery patches
May 11		5.5	6	As on Apr. 28
Apr. 28	OH 3 (1)	3.0	2	Healthy
May 11		8.0	3	Three leaves with weak powdery patches
Apr. 28	" (2)	5.0	3	Two leaves with powdery patches, one measuring $4 \times 3$ mm.
May 11		10.5	5	Five leaves with powdery patches up to 4 mm. diam.; powdery patch on stem 4 mm. long
Apr. 28	" (3) (a)	8.0	4	Two leaves with powdery patches
May 11		22.0	7	Five leaves with powdery patches
Apr. 28	" (b)	7.0	4	Two leaves with powdery patches, one measuring $5 \times 4$ mm.
May 11		13.0	6	Four leaves with powdery patches, including one with eight patches, $2 \times 2$ mm. or less
Apr. 28	" (4)	3.5	2	One leaf with minute powdery patch
May 11		10.0	4	Four leaves with small powdery patches
Apr. 28	" (5)	6.0	3	Healthy
May 11		12.0	5	Two leaves with small powdery patches
Apr. 28	" (6) (a)	1.5	2	Healthy
May 11		16.0	4	Four leaves with small powdery patches
Apr. 28	" (b)	4.5	1	Healthy
May 11		12.5	3	Two leaves with small powdery patches
Apr. 28	" (7)	6.5	2	Healthy
May 11		27.0	4	Six leaves with small powdery patches; one leaf at lowest node with 17 minute powdery patches
Apr. 28	OI 34 (1)	8.5	3	One leaf with two powdery patches; stem below 1st pair of leaves encircled by powdery patch 2 mm. long
May 11		27.0	6	Four leaves with powdery patches; patch on stem still powdery; powdery patch on petiole of one leaf
Apr. 28	" (2)	1.0	3	Healthy
May 11		2.5	4	One leaf with powdery patch, 2 mm. diam.
Apr. 28	" (3)	1.5	2	Healthy
May 11		3.0	3	One leaf with young mycelial patch

surrounded by mildewed plants in the greenhouse under conditions which ensured frequent inoculation by conidia, they all remained persistently immune. Here again, there appeared to be only a strictly limited period of susceptibility during which infection could take place.

Table III.

Date of exam.	Plant	Length of shoot (cm.)	No. of pairs of leaves	Condition
Apr. 28	310 (1) (a)	7.0	3	One leaf with minute powdery patch
May 11		9.0	4	Two leaves, each with minute powdery patches
Apr. 28	(b)	5.0	3	One leaf with minute powdery patch
May 11		7.0	4	One leaf with three minute powdery patches at margin
Apr. 28	" (2)	4.0	4	Healthy
May 11		4.5	4	"
Apr. 28	" (3) (a)	9.0	5	"
May 11		14.0	6	One leaf with two minute, scarcely powdery, patches
Apr. 28	(b)	8.0	4	Healthy
May 11		12.0	6	"
Apr. 28	" (4)	6.0	3	"
May 11		10.0	6	"
Apr. 28	" (5)	10.0	4	One leaf with very minute powdery patch
May 11		10.5	5	Leaves healthy; minute powdery patch dying away
Apr. 28	" (6) (a)	8.0	3	Healthy
May 11		19.0	4	One leaf with very minute powdery patch at margin
May 11	(b)	3.5	2	Healthy
Apr. 28		6.0	4	"
May 11	OJ 12 (1)	8.0	5	"
Apr. 28	" (2) (a)	14.0	3	"
May 11		24.0	5	Two leaves each with three slightly powdery patches
Apr. 28	(b)	10.0	2	Healthy
May 11		19.0	3	One leaf with weak powdery patch
Apr. 28	(c)	4.0	2	Healthy
May 11		7.0	3	"
Apr. 28	" (3) (a)	7.0	4	"
May 11		9.5	5	"
Apr. 28	(b)	3.0	2	"
May 11		5.0	4	"
Apr. 28	" (4)	9.0	5	"
May 11		11.0	6	"
Apr. 28	" (5)	6.5	3	"
May 11		8.0	5	"
Apr. 28	" (6)	10.5	4	"
May 11		29.0	7	One leaf (at the 2nd node from the base) with a yellowish area where a weak growth of scattered conidiophores occurred*

\* The term "sub-infection" has been used to denote cases in which inoculation is followed by the production merely of a few scattered conidiophores. See (7) (8).

## VARIETY 3. THE SEEDLING HOP, Ref. No. 310.

*Origin.* This is the same as that given above for the variety X 91.

*Evidence as to Immunity.* Four cuttings taken in 1919 were grown in pots in 1920 in a greenhouse where they remained immune, although continually exposed throughout the growing season to inoculation by conidia from surrounding mildewed plants.

*Partial breakdown of Immunity in 1926.* The material on which observations were made consisted of (1) six cuttings in pots taken in the autumn of 1925 from the original plant of Ref. No. 310, and six cuttings in pots of the "clone plant," Ref. No. OJ 12, both growing in the Experimental Hop Garden at Wye College; and (2) eight rooted plants raised at East Malling during 1925 from cuttings taken from the clone plants of Ref. No. 310 growing at the Research Station, East Malling, and transferred to pots at Wye during the winter of 1925. Both (1) and (2) were kept in an unheated greenhouse at Wye during the winter of 1925-6 and the spring and summer of 1926.

(1) The examination of these plants on April 28th and May 11th gave the results shown in Table III.

(2) These plants were examined only on April 28th, with the following results:

Table IV.

Plant	Length of shoot (cm.)	No. of pairs of leaves	Condition
310 (1) (a)	55.0	12	One leaf (at 7th node from tip) with very minute powdery patch
(b)	15.0	6	One leaf (at 3rd node from tip) with large powdery patch
„ (2)	90.0	—	All leaves healthy*
„ (3)	90.0	—	Large, very powdery patch (4 × 4 mm.) on one leaf (5th node)
„ (4)	80.0	—	Powdery patches on two leaves (4th node)
„ (5) (a)	100.0	—	All leaves healthy
(b)	60.0	—	„
„ (6) (a)	60.0	—	„
(b)	20.0	—	„
„ (7) (a)	60.0	—	„
(b)	30.0	—	„
„ (8)	30.0	—	Two leaves (at 2nd node from the base) each with a small powdery patch

\* The plant was used in Exp. 7. (See p. 272.)

The plants of (1) and (2) were kept through May and June in a greenhouse where they were exposed constantly to infection by conidia from contiguous plants covered with the mildew. No further infections

resulted. Here again, for the third time, the phenomenon of susceptibility was only temporary.

In one set of grafting experiments (see below, p. 281) scions of the variety 310, grafted on two different stocks, were inoculated, on May 28th and June 8th, with conidia taken from the very susceptible seedling hop, Ref. No. B 18. No infection resulted. In another set of grafting experiments, the variety 310 was used as the stock with grafts of a "wild hop." Inoculations on May 18th, June 3rd and 11th, with conidia taken from B 18 resulted in full infection of the grafts, while the leaves of 310 proved completely immune. The grafted plants were placed in the greenhouse where they were exposed to constant inoculation during the summer, but no infection resulted on the stocks or scions of the variety 310.

Two hypotheses may be advanced to account for the presence of the mildew on these "immune" plants: (1) the evolution of one or more specialised forms of *S. Humuli* able to attack "immune" varieties; (2) the effect of abnormal growing conditions either on (a) the host plant, so that its cells temporarily lost the power of preventing the fungus from infecting them, or on (b) the fungus, so that its spores temporarily acquired an increased power of infection.

On a *priori* grounds, the first hypothesis has much to support it. The phenomenon of specialisation of parasitism is found to a high degree in the Erysiphaceae. For example, in the species *Erysiphe Graminis*, the specialisation has proceeded to such an extent that we find<sup>(8)</sup> not one, but several specialised forms with distinctive powers of infection towards different species of the host genus *Bromus*. Experiments have shown<sup>(6)</sup> that the form of *S. Humuli* on species of *Humulus* is specialised to this genus. Further, Steiner<sup>(10)</sup> has shown that a number of specialised forms of *S. Humuli* exist on *Alchemilla* with distinctive powers of infection towards different species. It would not be unreasonable to suppose that, similarly, several specialised forms may evolve from the form on the genus *Humulus*, able to attack hitherto immune varieties. The circumstances may be conceived as being favourable for this. The immune plants were surrounded by susceptible plants heavily infected with the mildew. If among the millions of conidia being produced on these, one conidium varied in the direction of being able to infect an immune plant, and if then the conidia produced on this plant showed the same infective powers, a new specialised form would have arisen. In the Uredineae, the careful work of Stakman and his collaborators has shown<sup>(11)</sup> that specialisation of parasitism has taken place to a

remarkable degree; the specialised form *Tritici* of *Puccinia Graminis* has been found to be composed of a large number of further specialised forms with distinctive powers of infection towards different varieties of wheat.

With regard to the occurrence of mildew on the "immune" varieties of hops described above, evidence was sought as to whether a new specialised form might be evolving.

A few experiments were able to be made in which the infective powers of conidia produced on an "immune" variety were compared with those of conidia taken from a susceptible variety.

*Inoculation experiments with conidia taken from  
"immune" varieties of hops.*

*Exp. 4.* April 29th. Conidia were taken from the powdery patches produced on the plants OH 3 (2) and (3) of the variety X 91 (see above, Table II), and deposited on the two leaves (not quite fully expanded) of the 2nd node (from the tip) of a shoot 52 cm. long of a plant of the Golden Hop which for several years past had been grown in a pot in the greenhouse and had proved immune. On May 10th one of the inoculated leaves showed a number of scattered groups of conidiophores over the inoculated area; on the other inoculated leaf a very few scattered or more or less clustered conidiophores occurred at the place of inoculation—in both cases the appearance was that of "sub-infection" only. As, however, at this date the leaves at the next lowest node showed similar minute spots of mildew, but rather more powdery, the evidence as to the origin of the mildew on the inoculated leaves must remain doubtful. By May 31st, the little patches on the inoculated leaves were still evident but had not perceptibly increased in size; no mildew appeared on the leaves of the nodes higher up. On June 16th, the powdery patches began to die away, exposing to view minute raised brown patches of leaf cells. Such "injury spots" are characteristic of attacks by the mildew on the leaves of "semi-immune" plants (see (3), p. 257). No mildew occurred on the 22 leaves eventually produced above the inoculated leaves. If the mildew produced in this experiment on the inoculated leaves be considered as the result of the inoculation, this would be no evidence that the conidia produced on X 91 had any exceptional powers of infection, since weak infection of the Golden Hop can sometimes be produced by conidia taken from fully susceptible plants (B 18 and an unlabelled seedling) (see above, p. 265).

*Exp. 5.* May 7th. One leaf at the 2nd node from the tip of a shoot of the Golden Hop<sup>1</sup> was inoculated with conidia taken from "immune" plants of X 91 (OI 34, pot 1; OH 3, pots 2 and 3 (see Table II, p. 267)) and the other leaf at the same node with conidia from the fully susceptible variety B 18. Each leaf was inoculated in a drop of water at three marked places and when the water had dried, the deposited mass of spores was plainly visible. No infection resulted on either leaf. At the time of inoculation all the leaves of this plant of the Golden Hop were believed to be free from mildew; on May 17th, three very minute powdery patches were visible on one leaf near the base of the shoot (possibly overlooked on May 11th); these patches

<sup>1</sup> This plant was pot 2, in Table I (p. 264).



had increased slightly in size by the 21st, but no spread of the mildew took place and by the 31st, they had died away.

*Exp. 6.* May 25th. Two leaves (one-quarter expanded) at the 2nd node from the tip of shoots of two plants (pots 2 and 4) of the immune variety OH 3 (= X 91) were inoculated, one leaf with conidia taken from OH 3, pot 5 (see Table II) and the other leaf with conidia from a plant of the susceptible variety B 18. Water only was placed on the control leaves at the same node. No infection resulted in either case.

*Exp. 7.* April 30th. Two leaves at the 3rd node from the tip of a shoot of the rooted set (pot 2) of the immune variety 310 (see Table IV) were inoculated, one leaf at a marked place near the base of the centre lobe, with conidia produced on the leaves of another rooted set (pot 1) of 310 (see Table IV) and the other leaf, at three marked places (one on each lobe) with conidia taken from B 18. No infection resulted on either leaf.

*Exp. 8.* May 17th. One leaf (partly expanded) at the 2nd node from the tip of a rooted set (pot 6) of the variety 310 (see Table IV) and one leaf at the 2nd node of a plant of B 18, were heavily inoculated with conidia taken from the "immune" variety X 91 (OH 3, pots 2, 3, 4, 5, 7; see Table II). The opposite leaf at the node was treated with water only. By May 25th the leaf of B 18 was fully infected and by June 2nd, bore patches of densely clustered conidiophores "powdery" with accumulated conidia; the control leaf remained healthy. The inoculated leaf of 310 remained uninfected; the places where the conidia had been deposited were marked by little areas of dead brown epidermal cells—the typical reaction shown by the leaf of many varieties of hops immune from mildew.

It will be seen that the experiments detailed above give no evidence that the conidia produced on an "immune" plant possess distinctive powers of infection indicative of the evolution of a new specialised form. In this respect, the results obtained in *Exp. 8* seem conclusive. It is an interesting fact that a conidium produced on an "immune" plant may be unable to infect its host<sup>1</sup>, and one that suggests that the plant in question must have passed from a susceptible to an immune state.

The supposition, then, that the mildew on an "immune" variety might denote the advent of a new specialised form, finds no support from the above facts. Further evidence that these "immune" plants were not serving as the birth-place of new specialised forms is found in the fact that, as mentioned earlier, the mildew died away on all of them after a few weeks, without spreading to fresh leaves, under conditions in which the mildew attacked virulently the new leaves produced on ordinarily susceptible plants.

<sup>1</sup> We may compare the results of these experiments with those obtained where a plant has been artificially rendered susceptible to the attacks of the "wrong" specialised form of the mildew by the use of heat, alcohol, ether and other agents (see (9)). Conidia produced on such plants are not able to infect an untreated plant.

Nevertheless one may speculate whether in such cases as the above, where a biologic form of the mildew is able to live for a time on a host plant which normally is completely resistant, may not represent the first step in the evolution of a further biologic form adapted to certain members of the host genus. Possibly under a continuance of those special circumstances (which were probably weather conditions) which would enable the mildew to exist for a longer time on the new host plants, the conidia produced on them would possess the power of infecting the host plant under normal weather conditions. Of that, however, we have as yet no evidence.

We will now consider the second hypothesis, viz. whether there is any evidence for attributing the temporary susceptibility shown by the immune plants to abnormal growing (weather) conditions, the actual existence of which is indicated by the figures given below. The days when the first occurrence of mildew on immune plants was noted (April 28th) were dark and cold and the growth of the plants in the greenhouse either stopped entirely or there was only a very slight elongation of the stems. Unfortunately, figures of the temperatures in the greenhouse are not available. Mr A. H. Bird, Meteorological Observer at Wye College in connection with the Ministry's Agricultural Meteorological Scheme, has kindly supplied me with the chart showing the temperature of the air in the open at Wye for April and May, 1926. It

*Temperature of the Air. Mean of Daily Values (° F.).*

1926 Week ending	England. South-Eastern Counties				Wye, Kent			Deviation from normal for S.E. England*
	Max.	Min.	Mean (adjusted)	Deviation from normal*	Max.	Min.	Mean (adjusted)	
Apr. 3	57.6	39.0	48.0	+4.0	59.7	35.5	47.0	+3.0
" 10	57.8	43.5	50.3	+4.7	58.0	44.6	51.0	+5.4
" 17	55.8	39.8	47.4	+1.5	57.0	35.9	45.9	0
" 24	52.5	39.2	45.5	-1.4	51.6	37.7	44.3	-2.6
May 1	55.1	45.5	50.1	+1.6	55.3	44.3	49.4	+0.9
" 8	54.4	40.8	47.2	-2.2	51.7	41.1	46.1	-3.3
" 15	54.8	41.9	48.0	-3.1	54.1	41.1	47.2	-3.0
" 22	58.5	42.9	50.2	-2.5	58.0	41.3	49.1	-3.6
" 29	65.3	51.0	57.8	+3.9	67.6	49.6	58.0	+4.1
June 5	60.9	49.0	54.5	-1.3	61.0	48.0	54.1	-1.7
" 12	63.2	50.6	56.4	-0.5	61.7	49.3	55.1	-1.8

\* The "normal" is the mean for a 35 year period, and as regards Wye, is non-existent. The figures are not shown in the above table, but are arrived at by adding or subtracting the figures in the "deviation" column to or from those in the "adjusted mean" column.

will be observed that in the week ending April 24th, the minus deviation from the normal was 2.6 degrees, and those for the weeks ending May 8th, 15th and 22nd were all over 3 degrees. There does seem to be some evidence, then, that one factor influencing the temporary loss of immunity noted in 1926 may have been abnormal growing conditions. Other evidence exists, however, with regard to cases of loss of immunity noted in previous seasons, which must be considered here. In 1917, the seedling variety OR 38, which previously had shown in the greenhouse complete resistance to the mildew, exhibited slight and temporary susceptibility. This phenomenon was observed twice. In the first instance an inoculated leaf became weakly infected. The weather conditions were abnormal at the time (March, 1917); rapid changes of temperature occurred and the stem of the plant made scarcely any growth. Here it would seem reasonable to attribute the breakdown of immunity to the effect of weather conditions. In another case, however, susceptibility occurred under different circumstances. A leaf of OR 38 was heavily inoculated with conidia, together with leaves of two susceptible varieties; the latter became infected and OR 38 proved immune. On the 16th day after inoculation (May 22nd, 1917) a "control" leaf (at the 5th node from the tip) of the stem of OR 38 bore one minute powdery patch of mildew, the inoculated leaf, as well as all the other leaves on the stem being at this date and subsequently, entirely free from the mildew. Conidia taken from the patch failed to infect a young leaf of OR 38. Further details will be found in the account published in 1919 (2), pp. 84, 85). The conclusion then reached was that an immune plant in the greenhouse may show strictly local susceptibility without the general immunity being lost, and that this susceptibility was due to unknown causes. A similar and unexplained case of local susceptibility occurred in the open in 1918. A plant of the immune variety OB 34 (see (3), p. 256) growing in the Experimental Hop Garden at Wye College, produced a crop of some hundreds of healthy "hops" (strobiles), except for one young "hop" which alone was smothered with the conidial stage of the mildew.

Such cases as the above<sup>1</sup>, incline one to suspect that some other factor besides weather conditions exists which is able to cause localised susceptibility. On the other hand, again, there is the case already recorded, of a group composed of a number of different seedling varieties of hops which when grown in the greenhouse, remain season after season

<sup>1</sup> There is also the case of the Golden Hop in 1917, where as already mentioned above (p. 263) abnormal weather conditions had no effect upon its immunity.

completely resistant to the mildew but which, when grown in the open, sometimes prove at the end of the summer, to be susceptible, occasionally even to the highest degree<sup>1</sup>. A detailed history of the varieties of this group has already been given<sup>2</sup>. Here, again, it appears that the susceptibility is induced by special weather conditions and not by new specialised forms of the fungus.

#### SUMMARY.

1. Three varieties of hops which had previously shown complete resistance to the attacks of *S. Humuli*, became slightly infected in the greenhouse in the spring of 1926.

2. The susceptibility shown was only temporary; all the infected plants during May and June again acquired their power of complete resistance.

3. The conidia produced on an "immune" plant proved unable to infect that plant, although able to infect a susceptible variety.

4. It appears probable that abnormal weather conditions (possibly low temperature) brought about the temporary susceptibility.

#### BIBLIOGRAPHY.

- (1) SALMON, E. S. (1917). On Forms of the Hop (*H. Lupulus* L.) resistant to Mildew (*Sphaerotheca Humuli* (DC.) Burr.). *Journ. Agric. Sci.* VIII, 455.
- (2) — (1919). *Idem* II. *Journ. of Genetics*, VIII, 83.
- (3) — (1919). *Idem* III. *Ann. App. Biol.* v, 252.
- (4) — (1920). *Idem* IV. *Ibid.* VI, 293.
- (5) — (1921). *Idem* V. *Ibid.* VIII, 146.
- (6) — (1907). Notes on the Hop Mildew (*S. Humuli* (DC.) Burr.). *Journ. Agric. Science*, II, 330.
- (7) — (1904). On Specialisation of Parasitism in the *Erysiphaceae*. II. *The New Phytologist*, III, 111.
- (8) — (1904). On *Erysiphe Graminis* and its adaptive parasitism within the genus *Bromus*. *Annal. Mycolog.* II, 255.
- (9) — (1905). Further cultural experiments with "Biologic Forms" of the *Erysiphaceae*. *Annals of Botany*, XIX, 125.
- (10) STEINER, J. A. (1908). Die Spezialisierung der Alchemillenbewohnenden *Sphaerotheca Humuli* (DC.) Burr. *Centralbl. f. Bakt. etc.* XXI, 677.
- (11) STAKMAN, E. C. and LEVINE, M. N. (1922). The determination of biologic forms of *Puccinia Graminis* on *Triticum* spp. *Minn. Agr. Exper. Station Tech. Bull.* 8.

<sup>1</sup> In the case of one of these seedlings, Z 25, a few small patches of mildew appeared in 1919 on a few of the young leaves on Aug. 7th after a spell of abnormally cold, dull weather; these patches soon died away and in October the plant was entirely free from mildew. (See (5), p. 152.)

<sup>2</sup> (1), p. 457; (2), pp. 84, 85, 87; (3), pp. 254-8; (4), pp. 298, 306; (5), pp. 150-158.

## GRAFTING EXPERIMENTS WITH VARIETIES OF HOPS RESISTANT TO THE HOP POWDERY MILDEW, *SPHAEROTHECA HUMULI* (DC.) BURR.

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(With Plate XVIII.)

THE experiments on immunity described below, in that they have as an essential feature the employment of grafting, are an attempt to further the work of Roach<sup>(1)</sup> who has so successfully used this method for the investigation of the problem of potato immunity from wart disease (*Synchytrium endobioticum*). This author has demonstrated that when varieties of potatoes, resistant or susceptible to the attacks of *Synchytrium endobioticum*, are used as stocks and grafted with scions of varieties showing the opposite reaction to this fungus, the tubers produced are not influenced as regards their behaviour to the parasite.

Under the stimulus of the announcement of the above discovery, we were led to experiment firstly with the grafting of the hop plant and, secondly, with the effect of grafting on the immunity or susceptibility of the scion to the attacks of the hop mildew, *Sphaerotheca Humuli* (DC.) Burr. In 1925 we showed<sup>(2)</sup> that, using a method similar to that employed by Roach, the actual process of grafting the hop plant was not difficult. In the present paper the results are given of inoculation experiments made with conidia of the hop mildew, *S. Humuli*, on immune and susceptible scions respectively.

The only alteration in technique from that previously described<sup>(2)</sup>, in the process of grafting employed in these experiments consisted in the use of a trace of rubber solution to secure the last fold of the thin india-rubber tape which was the only binding material around the graft. The cleft method of grafting alone was used and the stock was cut at about 2 inches from the ground. No elongation of the stock was ever noticed to take place and the growth of the scion was therefore measured from the ground level. (See Tables I and II.)

There were two ways in which the relation of the composite plant to the mildew might be investigated: by grafting (1) susceptible scions on immune stocks, and (2) immune scions on susceptible stocks.

(1) In the first case any substance present in the stock, concerned in conferring immunity, might, if translocatable, be expected to influence the susceptibility of the scion; or again, if susceptibility be due to the presence of some substance found in susceptible stocks but absent from the immune stocks, then the cessation of supply might induce some degree of immunity.

(2) The same two considerations apply equally, *mutato mutando*, when immune scions are grafted on susceptible stocks. Both of the above types of graft were made.

After having been grafted, the whole plant or sometimes only the shoot concerned, was kept covered by a bell-jar or plugged lamp-glass for ten days. The grafted plants were placed in a shaded part of the greenhouse, out of direct sunlight, for the whole of that period and for a day or two after the glass covering had been removed. The rubber binding was then pulled away. At the time of grafting or within the following month, every internode of the grafted shoots was measured and the state of development of the leaves noted. In this way it was possible to determine, before selecting the plant for inoculation, not only whether growth had taken place, but also which pairs of leaves had unfolded from the bud during a part of the time that the scion had been receiving nourishment from the stock. Infection tests were only made upon leaves which had developed at some time subsequent to the date of the first of these records; any leaves which had then even been distinguishable (*i.e.* with tips projecting from the terminal bud) were neglected for this purpose. In most cases very considerable growth took place before any infection experiments were made and often the leaves used for inoculation were situated at a distance of four or five new internodes above the position occupied by the terminal bud at the time when the preceding record was made.

The varieties of hops used in the experiments, were as follows:

#### CLASS 1. IMMUNE VARIETIES.

1-3. "*Golden Hop*"; *seedlings Ref. Nos. X 91 and 310*. The facts concerning the origin and immunity of these three female varieties have already been given in a paper by one of us (see above, p. 264). Under normal circumstances, these three varieties are completely resistant to the attacks of *S. Humuli*.

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4-5. *Ref. Nos. II 124 and V 93.* These two male varieties were raised in 1913 from seed of a wild plant or plants of *Humulus Lupulus* obtained from Italy. As has been pointed out (3), both these varieties, when grown in the greenhouse, are completely resistant to the attacks of *S. Humuli*, although No. V 93, when grown in the open, may show a slight degree of susceptibility in the late autumn.

6. *Unnamed Immune.* This plant was one of a number of immune seedlings raised from the seed of wild *H. Lupulus* obtained from Italy, which had shown in the greenhouse for a number of seasons complete resistance to *S. Humuli*. Owing to a label in the pot having been lost, its further identification could not be established<sup>1</sup>.

### CLASS 2. SUSCEPTIBLE VARIETIES.

1-3. "*Wild Hop*," *A, B and C.* These were cuttings taken from (uncultivated) hops found growing wild in the hedge in three different localities at Wye<sup>2</sup>.

4. *Tutsham.* This is a commercial variety commonly cultivated in Kent; its degree of susceptibility to *S. Humuli* is unknown.

5. *Ref. No. B 18.* This is a variety of unknown parentage; it has been named the "Foundling" and has been described in the *Journal of the Board of Agriculture*, May, 1915. It has proved extremely susceptible in the greenhouse; occasionally its shoots, soon after their appearance above ground, become completely mildewed all over their surface and then hypertrophied.

### METHOD OF INOCULATION.

The powdery mildew of the hop (*S. Humuli* (DC.) Burr.) forms on the surface of the leaves (and occasionally on the stems) of susceptible varieties of hops, small or large mycelial patches on which densely-clustered conidiophores, bearing chains of conidia, are produced. Under favourable conditions for growth and in still air, the patches become "powdery" or "mealy" from the accumulation of ripe, detached conidia which have been produced successively in the greatest profusion.

In the experiments described below, the method of inoculation was as follows, unless otherwise stated. The leaf to be inoculated was moistened on the upper surface with distilled water. Using a small, clean camel-hair brush, conidia were placed in the film of water; the water evaporated in a short time and left the conidia deposited on the

<sup>1</sup> Very possibly the plant was *Ref. No. OB 34.* (See (3), p. 153.)

<sup>2</sup> In the South of England, such plants are believed to be not truly wild but to originate from cuttings of commercial varieties.

epidermis. The plants were in a greenhouse by themselves and were not covered over; the uninoculated leaf at the same node, or the remaining leaves on the plant, served as controls.

Except in the case of Exp. 1 *a* (Table I) the conidia used for inoculation were taken from clone plants of the variety B 18. As the possibility of the existence of a specialisation of parasitism was being explored (see above, p. 271), notes were kept of the exact source of the conidia used in each experiment but as no evidence was obtained that the infective powers of the conidia produced on different clone plants of B 18 varied in any way, these details are not given here. Seventy-six leaves of susceptible varieties (scion or stock) were inoculated and 72, *i.e.* 94.7 per cent., became infected.

### RESULTS OF EXPERIMENTS.

#### *Class 1. Scions of Immune Varieties grafted on Susceptible Stocks.*

Scions of six different immune varieties were grafted on stocks of four different susceptible varieties. The details of the grafting and the results of the inoculations are given in Table I and further particulars relating to the growth of the mildew in each experiment are given on p. 280. It will be seen that with the exception of the Golden Hop, where "sub-infection" occurred in two experiments, the immune scions remained immune. A typical case may be taken as illustration. In Exp. 7 *a* (see Table I) a scion of the immune variety V 93 was grafted, on March 12th, on the susceptible stock B 18. By March 29th the scion measured (from the ground) 15.6 cm. and possessed four pairs of leaves above the graft. On May 5 inoculations were made on leaves at the 8th and 9th nodes above the graft, the scion now reaching 79.0 cm., or more than five times its original height. The scion completely resisted infection. The same scion was again used on May 21st, by which time it had grown to 115 cm., more than seven times its original height; leaves at the 12th and 13th nodes above the graft were inoculated and proved to be completely resistant. In Exp. 11 the same scion was inoculated on three occasions on leaves at the 5th, 6th, 8th, 9th, 14th and 15th nodes above the graft, the scion being finally more than twelve times its original height; on each occasion the scion proved to be immune. In the case of the Golden Hop, the "sub-infection" which resulted in May on the four inoculated leaves in Exps. Nos. 1 *a* and 2 *a* was exactly similar to that which had been observed on ungrafted plants during April and May, 1926, and cannot therefore be ascribed to the influence of the susceptible stock. It will be noted that each scion was again inoculated after fresh growth had been made (Exps. Nos. 1 *b* and 2 *b*), and that they proved completely resistant.

#### *Class 2. Scions of Susceptible Varieties grafted on Immune Stocks.*

Scions of four different susceptible varieties were grafted on stocks of five different immune varieties. The results of the inoculations given in Table II and the particulars of the experiments given on p. 283 show that all the scions retained their original degree of susceptibility.



## DETAILS OF GRAFTING EXPERIMENTS.

1. *Immune Scions on Susceptible Stocks.* (See Table I.)

*Exp. 1 a.* Two leaves at the 3rd<sup>1</sup> node of the scion (Golden Hop) were inoculated, one with conidia taken from a plant of B 18, and the other with conidia from an unnamed seedling hop. The leaves were very young and only partly expanded; the conidia were placed in drops of distilled water on each of the three lobes of the leaf. Four leaves, at the 3rd and 4th nodes of the stock<sup>2</sup> (Wild Hop C), were similarly inoculated. On the 11th day all the four leaves of the stock were infected and bore mycelial patches with conidiophores; no infection was visible on the scion. By the 18th day the leaves at the 3rd node of the stock were almost continuously covered with mycelial growth, bearing dense powdery clusters of conidiophores; those at the 4th node were fully but less virulently infected. Of the inoculated leaves of the scion, one bore 2 conidiophores on its left lobe<sup>3</sup>, about 12 more or less isolated conidiophores and a cluster of 6 on the middle lobe, and a single conidiophore on the right lobe. The other leaf bore two minute clusters of 2 and 4 conidiophores, respectively, on the middle lobe. No mycelial growth was evident, and the general appearance was similar to that of "sub-infection<sup>4</sup>." No infection occurred elsewhere on the leaves of the scion.

*Exp. 1 b.* The above scion was used again 11 days later, by which time it had made a fresh growth of 16 cm. Two leaves at the 2nd and 3rd nodes of the scion, and similarly of the stock, were inoculated. By the 7th day there was abundant mycelial growth on the two inoculated leaves of the stock, and by the 11th day dense powdery masses of conidiophores; the control leaves at the same nodes were free from mildew. No trace of infection occurred on the two leaves, or others, of the scion.

*Exp. 2 a.* In the case of both scion (Golden Hop) and stock (Wild Hop C), four leaves at the 2nd and 3rd nodes were inoculated, the conidia being placed in a drop of water on each of the three lobes of the leaf. By the 10th day all the leaves of the stock were fully infected, and clusters of conidiophores were beginning to be formed. No signs of infection were apparent on the scion. By the 17th day the inoculated leaves of the stock were almost continuously covered with mycelial growth bearing powdery clusters of conidiophores.

Of the inoculated leaves of the scion, one at the 3rd node bore at two places on its right lobe minute clusters of 5-7 conidiophores; on its middle lobe, a few solitary conidiophores, while no infection occurred on the left lobe. As regards the other leaf at the 3rd node, a very few solitary conidiophores appeared on the right and middle lobes and no infection on the left lobe. At the 2nd node of the scion, one

<sup>1</sup> *I.e.* counting from the tip of the shoot

<sup>2</sup> In all cases, the inoculated leaves of the stock were not on the grafted stem but on a shoot coming from the same plant, since it was necessary to secure leaves of the same age and condition. Owing to their unsuitability in this respect, the leaves of the stock below the graft were never inoculated.

<sup>3</sup> The dorsal surface of the leaf was viewed with the petiole above.

<sup>4</sup> The term "sub-infection" has been used to denote the production of a few scattered conidiophores.

leaf showed 3 weak conidiophores on the left lobe and the other leaf a group of 5 conidiophores on the right lobe, 8 scattered conidiophores on the middle lobe and 3 isolated conidiophores on the left lobe. No infection occurred elsewhere.

*Exp. 2 b.* The above graft was used 19 days later when a fresh growth of 49.8 cm. had been made. One leaf at the 2nd and 3rd nodes of both the stock and scion were inoculated, the control leaf opposite being treated with water only. Full infection resulted on the stock, the control leaves remaining free; no trace of infection was observable on the scion.

*Exp. 3 a.* Four leaves at the 3rd and 4th nodes of the scion (Golden Hop) were inoculated, and two leaves at the 3rd node of a long stem of the stock (Wild Hop C) and two leaves at the 3rd node of a short shoot of the stock (of the same length as that of the scion). All the leaves of the stock became infected, and no infection resulted on the scion.

*Exp. 3 b.* The above scion was used 20 days later, when a fresh growth of 41 cm. had been made. Two leaves at the 2nd and 3rd nodes of the scion and two leaves at the 3rd and 4th nodes of the stock were inoculated, the control leaves being treated with water only. By the 22nd day the leaf at the 3rd node of the stock was somewhat weakly infected; no infection resulted on the leaf at the 4th node. The control leaves remained healthy. No trace of infection resulted on the scion.

*Exp. 4 a.* Two leaves at the 3rd node of both the scion (Golden Hop) and the stock (Wild Hop C) were inoculated. Infection occurred on the stock but not on the scion.

*Exp. 5 a.* Two leaves at the 2nd node of both the scion (No. 310) and the stock (Wild Hop A) were inoculated. The leaves were about one-quarter expanded. By the 7th day the leaves of the stock were fully infected; no infection occurred on the scion, but pale areas were visible at the places where the conidia had been deposited<sup>1</sup>.

*Exp. 6 a.* Two leaves at the 2nd node of both the scion (No. 310) and the stock (Tutsham) were inoculated. The leaves were about one-half expanded. By the 22nd day the leaves of the stock were fully infected, while no trace of infection occurred on the scion.

*Exp. 7 a.* Four leaves at the 2nd and 3rd nodes of the scion (No. V 93) were inoculated. No infection resulted. The stock was not inoculated in this experiment.

*Exp. 7 b.* The above scion was used 16 days later, when a fresh growth of 36 cm. had been made. Four leaves at the 2nd and 3rd nodes were inoculated. The leaves at the 2nd node were very small (1.5 to 2.0 cm. long) and at the 3rd node only about one-half expanded. No infection occurred. The stock was not inoculated in this experiment.

*Exps. 8 a and 9 a.* Two scions (No. II 24) on B 18 stock were used. Two leaves on an ungrafted shoot of the same stock were also inoculated. By the 16th day full infection had resulted on all three lobes of each of the two leaves of the stock; no infection occurred on the scion.

*Exps. 8 b and 9 b.* The two scions were again used 20 days later, by which time fresh growths of 57 cm. and 67 cm. (respectively) had been made. On one scion one leaf at the 2nd node was inoculated, and on the other one leaf at the 3rd. One leaf

<sup>1</sup> The same phenomenon has been observed when conidia have been deposited on the leaves of ungrafted resistant varieties.

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at the 3rd node of an ungrafted stem of the stock was also inoculated. All the inoculated leaves were about one-half expanded. The control leaves at the same nodes were treated with water only. By the 7th day complete infection had resulted on the stock, large mycelial patches (up to 0.5 cm. in diam.) bearing densely clustered conidiophores were present on all three lobes of the leaf; the control leaf remained healthy. No infection occurred on the scion; the inoculated leaves showed conspicuous, pale, often somewhat wrinkled areas where the conidia had been deposited.

*Exp. 10 a.* Four leaves at the 2nd and 3rd nodes of the scion (unnamed immune) and four leaves at the same nodes of the stock (No. B 18) were inoculated. By the 11th day the four leaves of the stock were all fully infected; by the 15th practically the whole of the upper surface of each leaf was continuously covered with the mycelial growth bearing conidiophores so dense powdery with accumulated conidia that a mist-like mass of conidia was dispersed through the air on the leaves being touched. No infection occurred on the scion.

*Exp. 10 b.* The same scion was again used 15 days later. Two leaves at the 2nd node were inoculated, as well as two leaves of the stock at the 2nd node. By the 11th day both leaves of the stock were almost continuously covered, on the upper surface, with mycelial growth bearing densely clustered conidiophores, already more or less "powdery" with accumulated conidia. No infection occurred on the scion; both the inoculated leaves showed pale areas where the spores had been deposited.

*Exp. 11 a.* Four leaves at the 2nd and 3rd nodes of the scion (unnamed immune), and four leaves at the same nodes of the stock (No. B 18) were inoculated. The leaves at the 2nd node of both scion and stock were about one-sixth expanded. By the 11th day the leaves of the stock at the 2nd node were fully infected and those at the 3rd node, weakly. By the 15th day the leaves at the 2nd node were so continuously covered with densely massed conidiophores bearing conidia, that a mist-like cloud of conidia dispersed on the leaves being touched; the leaves at the 3rd node bore more scattered but dense masses of conidiophores. No infection occurred on the scion.

*Exp. 11 b.* The scion was used again 16 days later by which time a fresh growth of 33.2 cm. had been made. Two leaves at the 2nd and 3rd nodes of both the scion and stock were inoculated. The leaves at the 2nd node were only partly expanded. The four control leaves at the same nodes were treated with water only. By the 8th day the leaf of the stock at the 2nd node was fully infected and showed over its surface an almost continuous mycelial growth, bearing densely clustered young conidiophores; the leaf at the 3rd node was infected with scattered mycelial patches and clustered young conidiophores. The control leaves remained free. No infection occurred on the scion. The inoculated leaves showed pale, slightly shrunken areas where the conidia had been placed—these were absent from the control leaves.

*Exp. 11 c.* The scion was again used 17 days later, when a fresh growth of 67 cm. had been made. Two leaves at the third node, and one leaf at the 2nd node of both scion and stock were inoculated. In both cases the leaves at the 2nd node were very small (7.8 mm. long); at the 3rd node they were about one-quarter expanded. The control leaf at the 2nd node was treated with water only. By the 13th day all the inoculated leaves of the stock were fully and heavily infected; the control leaf was healthy. No infection of the scion occurred and, as before, the inoculated leaves showed pale areas where the conidia had been deposited.

*Exp. 12 a.* Four leaves at the 2nd and 3rd nodes of both the scion (unnamed immune) and the stock (No. B 18) were inoculated. By the 11th day the four leaves on the stock were fully infected. No infection resulted on the scion.

*Exp. 12 b.* The above scion was again used 15 days later, when a fresh growth of 24.5 cm. had been made. Two leaves at the 2nd node of both the scion and stock were inoculated. By the 6th day the leaves of the stock were fully infected, whereas those of the scion were free.

*Exp. 13 a.* Four leaves at the 2nd and 3rd nodes of the scion (No. II 30) were inoculated. No infection resulted. It was noted that the scion made no growth during the experiment. No inoculation was made of the stock.

## 2. *Susceptible Scions on Immune Stocks.* (See Table II.)

*Exp. 1 a.* Four leaves of the scion (Tutsham) were inoculated at the 3rd and 4th nodes; those at the 3rd node were only half expanded. Two leaves of the stock at the 2nd node (Golden Hop) were inoculated. The leaves were moistened by spreading distilled water over them and conidia deposited over the damp surface; in the case of the leaves at the 4th node however, owing to the difficulty of getting the water to wet the surface of the older leaves, the conidia were deposited in drops of water. By the 7th day the two leaves at the 3rd node of the scion were seen to be fully infected; by the 13th day these leaves bore crowded and very powdery patches of conidiophores. No infection occurred on the leaves of the scion at the 4th node (6th above graft). This non-infection of the scion may be attributed possibly to the age and condition of the leaf. No infection occurred on the stock.

*Exp. 2 a.* Two half-expanded leaves at the 2nd node of the scion (Wild Hop B) and one leaf at the 2nd and 3rd nodes of the stock (No. 310) were inoculated, water only being placed in the latter case on the control leaf at each node. By the 7th day the two leaves of the scion were fully infected and by the 13th day bore very numerous dense powdery patches of conidiophores. No infection occurred on the stock; pale areas were observed on the epidermis where the conidia had been deposited.

*Exp. 2 b.* The above plant was used again 16 days later, by which time the scion had made a fresh growth of 85.0 cm. One leaf at the 2nd and 3rd nodes of the scion and the same also on a similar shoot of the stock were inoculated, the control leaves being treated with water only. The leaf at the 2nd node was only about one-quarter expanded. By the 14th day the leaf at the 2nd node of the scion was fully and heavily infected, and bore very numerous, dense powdery patches, while the control leaf was free; the leaf at the 3rd node remained uninfected, as also the control leaf at that node. No infection resulted on the stock.

*Exp. 3 a.* Four leaves at the 2nd and 3rd nodes of the scion (Wild Hop A), one leaf at the 2nd node, and two leaves at the 3rd node of the stock (No. 310) were inoculated. In the case of both stock and scion, the leaves were at the same stage of development, viz. at the 2nd node only just expanding and at the 3rd node about one-quarter expanded. By the 6th day all the four leaves of the scion were fully infected and showed an abundant mycelial growth and young conidiophores densely clustered. No infection occurred on the stock.

*Exp. 4 a.* Four leaves at the 2nd and 3rd nodes of the scion (B 18) were inoculated and by the 12th day were fully infected. No inoculation of the stock was made.

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*Exp. 4 b.* The above scion was used again 15 days later. Four leaves at the new 2nd and 3rd nodes were inoculated; the former were very small (1.5–2.0 cm. long), and the latter about half expanded. By the 10th day the leaves at the 2nd node were fully infected and those at the 3rd node weakly infected.

*Exps. 5 a and 6 a.* Two leaves at the 2nd and 3rd nodes respectively of the two scions (B 18) and one leaf at the 2nd and 3rd nodes respectively of the stocks (both unnamed immune) were inoculated. By the 7th day all the four leaves of the scions were fully infected. No infection of the stocks resulted.

*Exp. 7 a.* Four leaves at the 2nd and 3rd nodes of the scion (B 18) were inoculated. By the 19th day both the leaves at the 2nd node were so heavily infected with an almost continuous growth of dense powdery conidiophores that a cloud of conidia was dispersed on the plant being touched; the leaves at the 3rd node bore only small clusters of conidiophores spread over the surface of the leaf. No inoculation of the stock was made

### GENERAL DISCUSSION.

On the subject of the study of the nature of varietal immunity in plants, we may quote here the remarks made by Roach<sup>(1)</sup> with reference to varieties of potatoes immune from wart disease: "There is singularly little knowledge however of the general nature of this immunity. It may for example be conferred by a definite substance not present in the susceptible varieties, or on the other hand it may be due to the absence in the immune varieties of a substance which makes growth of *Synchytrium endobioticum* possible. If either of these suppositions be correct, this substance may be produced throughout the whole plant or it may be produced in one part and translocated thence to the remaining tissues. The possibility that the difference between 'immunes' and 'susceptibles' is the result of slightly different chemical groupings in the protoplasm in the two cases, does not seem to have been explored."

As regards immunity from *Sphacrotheca Humuli*, the results of our experiments (Tables I and II) in which a considerable number of composite plants were built up by grafting, viz. 7 plants of susceptible varieties grafted on immune, and 13 plants of immune varieties grafted on susceptible, show that the original degree, either of resistance or susceptibility, in the ten varieties used remained unchanged in the scion. No evidence was obtained of decreased infection of the susceptible scion when grafted on immune stocks. Conversely, shoots of immune varieties grafted on susceptible stocks remained immune. For both these types of experiment a variety such as B 18, with its known extreme susceptibility to the mildew, was very suitable and make the results obtained the more noteworthy. It is to be remarked further that among the immune varieties used, two, viz. Golden Hop and No. 310.

have been observed to show in the greenhouse a temporary breakdown of immunity which permits of the production of a weak growth of conidiophores on the leaves. These cases have been described above (p. 263) and the cause of the loss of immunity has been tentatively ascribed to the effect of weather conditions (possibly low temperature).

In the case of another immune variety used, viz. No. V 93, although the plants grown in the greenhouse have always shown complete resistance, a slight susceptibility in plants grown in the open has occasionally been observed in the late autumn (3), p. 151). Of these three varieties, scions of Nos. 310 and V 93 showed in the grafting experiments complete resistance to the mildew, while the scions of the Golden Hop remained immune in five of the experiments and in two became "sub-infected" in exactly the same manner as did certain ungrafted plants of that variety in the greenhouse<sup>1</sup>. Such plants as the above three varieties may be considered as especially suitable for testing the effects of grafting because an interference in their metabolism, such as may apparently be produced by low temperature, results in a temporary loss of complete resistance to the mildew. It is all the more interesting, therefore, to find that the process of grafting with the possibility of the scion experiencing an alteration in the supply of substances from a susceptible stock, affects in no way the varietal reaction to the attacks of the mildew.

#### SUMMARY.

1. Twenty composite plants were built up by grafting either a scion of an immune variety of hop on a susceptible stock, or *vice versa*. Inoculation of young leaves of the scion, after a considerable growth of the latter had taken place since grafting, showed that no change had been effected in the varietal immunity or susceptibility in relation to *Sphaerotheca Humuli*.

2. In the above experiments, scions of six different immune varieties were grafted on stocks of four different susceptible varieties and scions of four different susceptible varieties were grafted on stocks of five different immune varieties.

3. The results obtained render it improbable that immunity or susceptibility to *S. Humuli* is connected with any translocatable substance in the hop plant.

<sup>1</sup> See above, p. 263

Table I.  
*Grafting Experiments with Immune Scions on Susceptible Stocks.*

Exp. No.	Susceptible stock	Immune scion	Date of graft	Date of records	Total height of scion (cm.) from ground	Pairs of leaves expanded above graft	Node above graft where leaf inoculated	Distance of node from growing point (cm.)	Result of inoculation on Stock	Scion
1	Wild Hop C	Golden Hop	Mar. 27	Apr. 13	18.5	7	7th	8.5	+4	+2
(a)	"	"	"	" 29	27.5		(10th 11th	14.0) 8.0)	+2	{ -1 -1
2	Wild Hop C	Golden Hop	" 27	May 10	43.5	7				
(a)	"	"	"	Apr. 13	20.8		{ 7th 8th	14.5) 10.8)	+4	{ +2 +2
(b)	"	"	"	" 30	35.2		{ 13th 14th	23.0) 13.0)	+2	{ -1 -1
3	Wild Hop C	Golden Hop	" 27	May 19	85.0	6				
(a)	"	"	"	Apr. 13	13.2		{ 7th 8th	21.0) 17.6)	+4	{ -2 -2
(b)	"	"	"	May 5	39.0		{ 15th 16th	15.0 7.0	-1 +1	-1 -1
4	Wild Hop C	Golden Hop	" 27	" 25	80.0	6			+2	-2
(a)	"	"	"	Apr. 13	23.4		7th	6.6		
	"	"	"	May 5	31.0	3				
5	Wild Hop A	310	May 13	" 13	13.8		9th	15.0	+2	-2
(a)	"	"	"	June 8	55.0	4				
6	Tutsham	310	" 18	May 18	28.1		6th	12.5	+2	-2
(a)	"	"	"	" 28	47.5					

7	B 18	V 93	Mar 12	Mar 29	15.6	4	{ 8th 9th 12th 13th	{ 30.0 20.0 24.0 14.0	{ -2 -2 -2 -2
	(a)	"		May 5	79.0			(0)	(0)
	(b)	"		" 21	115.0			(0)	(0)
8	B 18	11 24	" 12	Mar 29	10.1	3	6th 10th	11.5 18.0	+1 +1
	(a)	"		May 5	32.0				
	(b)	"		" 25	89.0				
9	B 18	11 24	" 12	Mar. 29	10.4	3	5th 11th	13.6 12.0	+1 (0)
	(a)	"		May 5	33.0				
	(b)	"		" 25	100.0				
10	B 18	Unnamed immune	" 13	Mar. 29	15.5	4	{ 6th 7th 9th	{ 6.8 3.4 —	{ -2 -2 -2
	(a)	"		May 6	33.0			+4	+4
	(b)	"		" 21	46.0			+2	+2
11	B 18	Unnamed immune	" 13	Mar. 29	11.8	3	{ 5th 6th 8th 9th	{ 22.5 12.5 27.0 14.0	{ -2 -2 -1 -1
	(a)	"		May 6	41.8			+4	+4
	(b)	"		" 17	75.0			+2	+2
	(c)	"		June 3	142.0		{ 14th 15th	29.5 14.5	{ -2 -1
12	B 18	Unnamed immune	" 13	Mar. 29	27.4	4	{ 6th 7th 10th	{ 7.4 4.0 10.5	{ -2 -2 -2
	(a)	"		May 10	38.0			+4	+4
	(b)	"		" 25	62.5			+2	+2
13	B 18	11 30	" 12	Mar. 29	12.0	2	{ 4th 5th	{ 3.2 1.0	{ -2 -2
	(a)	"		May 7	24.4			(0)	(0)



Table II.  
Grafting Experiments with Susceptible Scions on Immune Stocks.

Exp. No.	Immune stock	Susceptible scion	Date of graft	Date of records	Total height of scion (cm.) from ground	Pairs of leaves expanded above graft	Node above graft where leaf inoculated	Distance of node from growing point (cm.)	Stock	Result of inoculation on scion
1	Golden Hop	Tutsham	Apr. 20	May 6	24.2	5	{ 6th 7th }	{ 24.5 17.0 }	-2	{ -2 +2 }
2	"	"	"	" 18	49.2					
(a)	310	Wild Hop B	" 15	" 6	24.8	7				
(b)	"	"	"	" 18	65.0		8th	24.1	-2	+2
3	"	"	"	June 3	150.0		{ 13th 14th }	{ 29.0 15.5 }	-2	{ -1 +1 }
(a)	310	Wild Hop A	May 13	May 13	23.5	5	{ 10th 11th }	{ 34.5 19.3 }	-3	{ +2 +2 }
4	V 93	B 18	Mar. 12	June 11	92.0	3				
(a)	"	"	"	Mar. 29	11.2					
(b)	"	"	"	May 5	49.0		{ 5th 6th }	{ 22.0 14.0 }	(0)	{ +2 +2 }
5	Unnamed immune	B 18	" 13	" 21	59.0	2	{ 8th 9th }	{ 12.0 7.0 }	(0)	{ +2 +2 }
(a)	"	"	"	Mar. 29	6.5					
6	Unnamed immune	"	"	June 10	58.0	3	12th	18.5	-1	+2
(a)	"	B 18	" 13	Mar. 29	16.8					
7	"	"	"	June 10	76.0	3	15th	9.0	-1	+2
(a)	II 30	B 18	" 12	Mar. 29	12.5					
"	"	"	"	May 7	58.2		{ 6th 7th }	{ 31.0 19.0 }	(0)	{ +2 +2 }



SALMON & WARE. GRAFTING EXPERIMENTS WITH VARIETIES OF HOPS (pp. 276- 289).



## BIBLIOGRAPHY.

- (1) ROACH, W. A. (1923). Studies in the Varietal Immunity of Potatoes to Wart Disease (*Synchytrium endobioticum* (Schilb.) Perc.). Part I. *Ann. App. Biol.* x, 142.
- (2) SALMON, E. S. and WARE, W. M. (1925). Virus Diseases and the Grafting of the Hop. *Gardeners' Chronicle*, May 9th, LXXVII. 320.
- (3) SALMON, E. S. (1921). On Forms of the Hop (*H. Lupulus*) resistant to Mildew (*S. Humuli* (DC.) Burr.); V. *Ann. App. Biol.* VIII, 150, 151, 156.

## EXPLANATION OF PLATE XVIII

The susceptible variety of Hop, B 18, with two shoots. Right, ungrafted; left, a shoot on which a scion of an immune variety was grafted at (a) on March 13th. After vigorous growth had taken place, two young leaves of the same age on each shoot were inoculated on May 21st. The immune scion has completely resisted infection (b), whereas the stock has become fully infected (c). Photographed June 1st. (See Table I, Exp. 10 b ) { Natural size.

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RHIZOCTONIA "FOOT-ROT" OF THE TOMATO<sup>1</sup>

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EPIDEMICS of foot-rot of young tomato plants caused by *Rhizoctonia solani* (Kühn) have inflicted considerable losses upon some growers in Guernsey. The disease appears shortly after planting in the houses and results in the formation of brown lesions at soil level which gradually extend and finally cause the plant to fall over. In the present investigation the effect of various cultural devices and also of soil fungicides has been tested.

## I. INOCULATION EXPERIMENTS.

These experiments were conducted in a glasshouse having an average temperature of 21° C. and tomato plants in four-inch pots were used. All factors were kept as constant as possible with the exception of that under consideration. Inoculations were made from cultures on Coons agar, the inoculum being so placed that the fungus was compelled to grow through half an inch of soil before reaching the plants. Cultures of the same age were used for all the plants in any one experiment; this precaution was necessary because a decided loss of virulence, indicated by meagre production of sclerotia, occurred in pure culture which was however regained by passage through a host plant. Controls received culture medium only and remained healthy in all cases. At the conclusion of each experiment, the duration of which varied from 7 to 14 days, the plants were examined and re-isolations were made from representative members. To estimate the extent of the disease the following degrees of infection were adopted: none; fair, lesion  $\frac{1}{4}$  in. to  $\frac{1}{2}$  in. long; bad, lesion  $\frac{1}{2}$  in. or more in length. Preliminary experiments, made with the object of finding a suitable variety for later work, showed that all the varieties tested were equally susceptible and as Ailsa Craig was available it was used for all subsequent experiments.

<sup>1</sup> Portion of a thesis approved for the Degree of Master of Science in the University of London.

1. *The relation of soil moisture and soil type to the disease.*

A knowledge of this relation is especially desirable because the amount of water supplied and the method of application can be varied at will under nursery conditions. Inoculated plants were submitted to different moisture conditions, the details of which with the results obtained are shown in Table I.

Table I.

Degree of infection	Daily treatment per four-inch pot			Soil type	
	No water	50 c.c.	100 c.c.	Sandy	Clay
None	21	2	2	6	3
Fair	8	11	3	5	—
Bad	1	17	19	6	14
Total No. of plants	30	30	24	17	17

These results suggested that planting in furrows as practised by some growers would render the plants more liable to infection and this was confirmed experimentally.

The above evidence indicated that the disease is least prevalent on dry, open soils and emphasised the danger of furrow planting.

2. *The effect of temperature upon the disease.*

To determine the optimum temperature for infection inoculated plants were placed in different positions, the maximum, minimum, morning and afternoon temperatures of which were recorded each day. The temperature variation in these positions was no greater than is usual in large glasshouses and does not therefore invalidate the practical value of the results. Consideration of the data given in Table II shows that severe infection occurred between 16.1° C. and 20.7° C. and later experiments confirmed that below and above these temperatures respectively, the degree of infection diminished.

Table II.

Temperature in ° C.			No. of plants	Degree of infection		
Absolute minimum	Absolute maximum	Average		None	Fair	Bad
19	42	27.0	21	13	6	2
11	34	20.7	21	6	7	8
9	24	16.1	21	1	2	18
2	12	7.4	15	13	2	—

Recent work at Cheshunt has shown that harmful results occur when tomato plants are set out at a soil temperature less than 13·8° C. (57° F.) with the result that most growers do not plant until the soil is sufficiently warm. Further, in commercial practice soil watering is withheld until the fruit on the second truss is swelling, a period of about 60 days after planting, because earlier watering is prejudicial to the setting of the earlier flowers. It follows that the soil must be reasonably moist when planting. Thus the temperature and moisture conditions of the soil at planting time are normally favourable to infection by *Rhizoctonia*. Growers have some difficulty in obtaining a soil temperature higher than 15·5° C. (60° F.) at planting time and indeed this temperature is attained only by heating the empty houses for 14 to 20 days previously. Therefore any control measure which depends upon soil temperatures higher than 15·5° C. is almost impracticable. It is also difficult to lower the moisture content of the soil at planting time but growers should remember that dry open soils are least favourable to *Rhizoctonia*.

### 3. *The effect of manurial treatment upon the disease.*

An attempt was made to see how infection by *Rhizoctonia* was affected by the various manures used in tomato culture.

The abundant fungal growth produced upon sterile stable manure suggested that this material might be expected to increase the amount

Table III.

Manure	Percentage in soil	No. of plants	Degree of infection		
			None	Fair	Bad
Stable manure	None	17	2	7	7
"	3·0	17	1	3	13
"	6·0	17	2	—	15
Ammonium sulphate	None	38	2	16	20
"	0·5	38	14	16	8
Complete artificials*	None	10	—	1	9
"	1·5	10	5	4	1
Complete artificials minus potash	1·0	10	2	5	3
Complete artificials minus nitrogen	1·0	10	—	1	9
Complete artificials minus phosphate	1·0	10	3	6	1

\* Complete artificials includes potassium sulphate, superphosphate and ammonium sulphate.

of infection and it is clear from the results of confirmatory experiments given in Table III that this is actually the case.

The results of the fertiliser experiments were not conclusive but suggested that a top dressing of sulphate of ammonia may reduce the amount of disease, and field experiments are being arranged to test this assumption on a larger scale.

Lime used in various quantities from 1 to 10 lb. per square yard scarcely affected the degree of infection.

## II. SOIL STERILISATION.

### 1. *Summer treatment.*

An attempt was made to discover a fungicide which would enable the grower to re-plant the infected areas at once. For this purpose solutions of the following compounds were tested using 100 c.c. of a strength which just failed to kill the plants: Cheshunt compound, copper sulphate, sulphuric acid, formaldehyde uspulun, ammonium polysulphide, ammonium hydroxide, ammonium carbonate, ammonium sulphate, ammonium chloride and ammonium nitrate. Only uspulun appeared to have any effect upon the disease and in a final test with this fungicide, using a 0.25 per cent. solution in sufficient quantity to saturate the soil, 13 out of 16 plants escaped infection whilst all the controls were severely diseased.

### 2. *Winter treatment.*

It is obvious from observation in commercial nurseries and experiments conducted during the course of this investigation that the organism causing this disease overwinters in the soil and therefore on infected nurseries it is necessary to sterilise the soil at the end of the season. Laboratory experiments sufficed to show that infected soil, even when large sclerotia were present, can be freed from *Rhizoctonia* by steaming for one hour at 96° C. and that when such soil is re-infected after sterilisation the disease spreads much more rapidly than in non-steamed soil. Baking in a standard oven gave similar results. Steam sterilisation of infected soils on nurseries has repeatedly given freedom from this disease.

Some growers cannot be persuaded to employ steam sterilisation because they live in localities where boilers cannot be hired and they do not wish to incur initial cost of the necessary apparatus. For this reason a number of likely chemical compounds was tested to see if the disease could be controlled by the use of chemical sterilising agents. For this purpose copiously infected soil was passed through a  $\frac{1}{4}$  in. sieve



prior to treatment with fungicides which were diluted at the rate of 100 c.c. per pound of soil to be treated and were applied as evenly as possible. Soil treated with volatile compounds was covered for two days. Controls received water instead of fungicide and when planted infection always occurred. The following compounds proved to be valueless: cresylic acid (0.36 per cent.), lysol (0.08 per cent.), potassium dichlorophenol (0.056 per cent.), copper sulphate (1.32 per cent.), flowers of sulphur (0.5 per cent.) and potassium permanganate (0.27 per cent.).

Promising compounds were tested again and the results, given in Table IV, showed that in no case was the soil freed from the disease although the treatment was such that it would be too costly for use on a commercial scale. When it is remembered that these experiments were carried out carefully on a small scale it seems reasonable to conclude that control by these fungicides is less reliable than steaming and is indeed rarely possible under nursery conditions.

Table IV.

*Sterilisation of infected soil by fungicides.*

Compound	Percentage in soil	Application per acre in tons	No. of plants	Number infected
Formaldehyde	0.250	2.50	10	3
Phenol	0.220	2.20	10	2
Potassium dichlorocresylate*	0.032	0.32	10	2
Potassium dichlorophenate*	0.065	0.65	15	3
Ammonium carbonate	0.880	8.80	15	4
Ammonium hydroxide	1.100	11.00	10	4

\* Viable sclerotia were found on the sides of some pots containing healthy plants

## III. CONTROL METHODS.

As a result of this investigation the following methods of control may be suggested:

1. Diseased plants and the soil immediately surrounding them should be removed, care being taken to ensure that no soil is transferred from the diseased area to other parts of the house, and the hole thus made should be treated with a 0.25 per cent. solution of uspulun. The area may then be re-planted and the plants given a top dressing of ammonium sulphate.

2. Dry open soil conditions should be maintained by periodic hoeing, and, if possible, by reducing the water supply and raising the temperature.

3. The soil should be steamed after uprooting and removing the plants at the end of the season.

4. Weeds in and about the glasshouses must be destroyed, as experiments have indicated that these may serve as hosts and thus help to maintain the virulence of the fungus.

The author wishes to acknowledge his indebtedness to Dr W. F. Bewley, Director of the Experimental and Research Station, Cheshunt, for advice and helpful criticism during the investigation and the preparation of this paper.

#### IV. SUMMARY.

1. A "foot-rot" disease of young tomato plants from Guernsey caused by *Rhizoctonia solani* has been investigated.

2. The disease is least prevalent on dry open soils; it is most severe at temperatures ranging from 16° C.-20° C.

3. The degree of infection was increased by stable manure; slightly decreased by ammonium sulphate; apparently unaffected by lime, potassic and phosphatic manures.

4. Soil sterilisation by heat was the most effective and reliable means of controlling the disease. During the season the disease was checked by uspulun.

5. Control methods are suggested.

(Received April 4th, 1927.)

## MANGEL SCAB—ITS CAUSE AND HISTOGENY

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(With Plates XIX–XXII and 1 Text-figure.)

### INTRODUCTION.

THE causative organism of mangel scab has long been recorded as identical with that of common scab of potatoes and the work of previous investigators leaves no room for doubt that the two diseases are closely allied. It is moreover a common experience to find that where potatoes are scabbed, mangels and beet and also turnips grow on the same soil are similarly affected. Since, however, Wollenweber<sup>(1)</sup> and Millard and Burr<sup>(2)</sup> have shown that potato scab may be produced by a variety of *Actinomyces* species, the assumption of identity between the two diseases becomes meaningless unless, indeed, it should transpire that each of the *Actinomyces* species producing scab on potatoes is able also to produce it on beet. It has also become obvious that, at the time of the early investigations of beet scab, no accurate comparison could be made between *Actinomyces* strains isolated from the two diseases. In this connection, however, it is interesting to note that in 1904, Krüger<sup>(3)</sup>, working on what he called "girdle" scab of sugar beet, stated that the *Actinomyces* strains which he isolated were not identical with the Thaxter organism of potato scab, and again, in 1915, Lutman and Johnson<sup>(4)</sup> recorded the isolation of eight strains of *Actinomyces* drawn from beet scab, five of which were found to be pathogenic. One of these was decidedly more virulent than the remaining four. In spite of these results, the identity of the organisms of potato and beet scab was tacitly accepted.

The morphological differences between the disease in its most noticeable forms on the two hosts are obvious even to the casual observer. In the potato the scabs are frequently sunken (pitted) or, if superficial,

consist of a mass of cork, whereas, in the beet the scab frequently forms large knob-like protuberances up to 20 mm. across and rising 6 mm. or more above the surface. It is plain that this morphological difference may be due either to different organisms or to innate differences between the two host plants in their reaction to the invading parasite, or again, to a combination of both factors. The present work was undertaken with the object of finding the causative organism or organisms of beet scab and of ascertaining more precisely the manner in which the scabs developed under their invasion.

### PRESENT WORK.

Mangels of the Golden Tankard and Red Intermediate varieties were obtained from a field at Strensall where the soil is a very sandy loam and yields badly scabbed crops of potatoes. The mangel crop was severely scabbed and photographs of typical roots are shown in Plate XIX, figs. 1 and 2. It will be observed that the scabs are in general confined to that portion of the root which is at or below soil level. Examination of these specimens revealed the fact that, in addition to the large bulging scabs, another type of scab was also present. This consisted of an irregular cavity filled with brown rotting tissue covered when young, by a plate of hard black suberised tissue. Other scabs in the neighbourhood of these shallow pits consisted of an irregular, corky mass rising slightly above the surface of the root in an uneven manner very different from the more noticeable bulging scab and may possibly have been older stages of the pitted variety. They are best seen in the specimen shown in Plate XIX, fig. 2.

### ISOLATION OF THE CAUSATIVE ORGANISMS.

#### (I) *Pitted Scab.*

The mangels were first thoroughly washed and allowed to dry. A typical scab was then selected and wiped with a camel hair brush dipped into methylated spirits. With the aid of a sterile scalpel the upper part of the scab was removed whilst the tissue below was mulched into a pulp. A small portion of this pulp served as inoculum and with it plates of nutrient potato agar were poured. The latter were incubated at 24° C. for 7 days and the colonies which had then grown in each dilution plate were found to be almost entirely *Actinomyces*. All the colonies appearing in the plates of the higher dilutions stained the

medium a dark brown and none produced aerial mycelium. As no differences could be observed between them pure cultures were made from only two; these eventually proved to be duplicates and form Strain 6 of this paper.

## (II) *Raised Scab.*

From subsequent work on the structure of raised scab it seems very possible that there are two variants of this type and these will be later described. At the time of making these isolations no such distinction had been drawn, but the inoculation material from which the cultures were obtained was taken from a bulging scab with gently sloping sides which afterwards came to be known as "mound" scab. The same procedure was followed as in the case of pitted scab, but in the first two attempts at isolation the plates showed large numbers of bacteria and very few *Actinomyces*. Finally, however, the inoculum was taken from the periphery of the scab and here the results obtained were much more satisfactory. Bacterial and Actinomycetal colonies were present in approximately equal numbers. None of the *Actinomyces* colonies stained the medium, but other variations were noticed between them and eight were isolated into pure culture. A preliminary examination of the growth of these was made on calcium malate glycerine agar, glycerine synthetic agar, dextrose synthetic agar, and nutrient potato agar, and by this means three of the eight strains were judged to be duplicates; the cultures were thus reduced in number to five (Strains 1, 2, 3, 4 and 5), all of which proved to be different from one another.

## INOCULATION EXPERIMENTS.

The task before us consisted first in testing Strain 6 (from pitted scab) and the five strains (Nos. 1-5) from knob scab *individually*, and for this purpose, it was thought desirable to use duplicate pots each containing three plants for inoculation with each strain. Secondly, it was necessary to carry out inoculations with as many *combinations* of the five strains (Nos. 1-5) as possible. It was obviously impossible to try all possible combinations since, if for each inoculation a maximum of only three roots were used, the number of plants required would have been too large to cope with. The multiple inoculations were therefore limited to the possible combinations taking two strains at a time. From previous experience with potatoes it seemed that the most satisfactory method of inoculation in such an experiment was that of soil inoculation, but, again in order to spare the labour involved, it was determined to

use this method only for the *individual* inoculations and to carry out the *dual* inoculations by direct application of the inoculum to the growing roots. It was thought that in addition to the inoculations so arranged, it would be of great interest to test the effect of a potato scab producing *Actinomyces* species on mangels and for this purpose the very virulent species *A. scabies* (Thaxter) Güssow, emend. M. and B., was selected and included in the series of direct inoculations. Finally, since before the direct inoculations were carried out it became obvious that Strain No. 6 was proving positive in the soil inoculations, a direct inoculation with this strain was also made.

For the soil inoculations 14 large plant pots were filled with soil which had previously been mixed with sand in the proportion of 2 parts loamy soil to 1 part sand. The mixture thus obtained was a light sandy loam in which *Actinomyces* are known to thrive. Its lime requirement was nil. The pots were sterilised at  $3\frac{1}{2}$  atmospheres' pressure for 15 minutes and were then transferred to a previously sterilised greenhouse where the temperature varied from  $18^{\circ}$  to  $20^{\circ}$  C. by day and from  $11^{\circ}$  to  $16^{\circ}$  C. by night. Inoculations of the soil were made with potato plug cultures of the various strains, using 20 cultures to each pot. These were buried to a depth of 4 in. and then cut and mixed with the soil by means of sterile scalpels.

The mangel plants required for the direct inoculations were grown in large boxes 6 ft. long by 1 ft. deep filled with unsterilised soil.

The varieties of mangel selected for both series of inoculations were Yellow Globe and Red Intermediate. The seed was first sterilised in a  $\frac{1}{6}$ th per cent. solution of formaldehyde for 2 hours after which it was washed in distilled water and dried under cover on a porous plate. In order to ascertain whether this treatment had affected the germination of the seed adversely, germination tests were carried out before sowing. Yellow Globe Mangel gave a germination capacity of 70 per cent. and Red Intermediate 55 per cent. In sowing the seed in the pots, therefore, five seeds were placed in each of three shallow holes in the soil of each pot, whilst, in the boxes similar sowings were made at distances of 8 in. apart. Each pot was given 15 gm. of a sterilised mixture of artificial manure consisting of 1 part sodium nitrate, 3 parts superphosphate and 3 parts kainit and the boxes were given proportionate quantities of the same mixture. When the seedlings were in their 3rd or 4th leaf they were singled, so that, finally, each pot contained three roots, one red and two yellow, and in the boxes similar groups of three were made by inserting thin strips of wood to a depth of 6 in. in the soil.

Direct inoculation of the plants in the boxes was carried out when the diameter of the bulbed roots was from 3 to 4 in. Each group constituted a unit for one mixed inoculation. The inoculum used was prepared by mixing emulsions made with sterile water of the two strains of *Actinomyces*. A band of damp cotton wool was tightly secured around each bulb at soil level and the emulsion was then poured on the root so that it lodged between the surface of the root and the cotton wool. The roots were not injured in any way before or after adding the inoculum. During the further period of the experiment the cotton wool around the roots was moistened from time to time with sterile water. It will be seen that in this procedure the possibility of natural infection of the roots from the soil was not excluded. One group of three roots was, however, left as control and here sterile water in place of an inoculum was poured between the cotton wool and the root. At the time of making the direct inoculations the roots were perfectly free from scab and no scab developed on the three controls during the experiment. Moreover, the great majority of these dual inoculations gave negative results and we feel justified, therefore, in accepting such as were positive. These were abundantly confirmed by the soil inoculation results.

It may be of interest to record that every plant left in the pots and

### *Results of Inoculation Experiments.*

#### *Series 1. Soil Inoculation.*

No. of pot	Inoculated with <i>Actinomyces</i> strain No.	Result
1	1	Negative
2	1	"
3	2	"
4	2	"
5	3	"
6	3	"
7	4	Positive. Producing mound scabs from 5 to 15 mm. diam. on Yellow Mangels only
8	4	Positive. Ditto
9	5	Negative
10	5	"
11	6	Positive. Producing small dark pitted scabs on both Red and Yellow Mangels and also forming numerous scab nodules on the true roots. Some superficial scab was shown on the upper part of one root
12	6	Positive. Ditto
13	Control	Negative
14	"	"

## Series 2. Direct Inoculation.

Group of roots No.	Inoculated with <i>Actinomyces</i> strain Nos.	Result
1	1 + 2	Negative
2	1 + 3	"
3	1 + 4	Positive. Very small raised scabs
4	1 + 5	Negative
5	2 + 3	"
6	2 + 4	Positive. A few minute scabs of indefinite type
7	2 + 5	Negative
8	3 + 4	"
9	3 + 5	"
10	4 + 5	"
11	6	Positive. One large pitted scab on the Yellow Mangel with numerous nodules on the upper rootlets
12	<i>A. scabies</i>	Positive. Small brown pitted scabs on all three roots

in the boxes after singling formed a good bulbous root which in some cases measured as much as 4 in. in diameter—this, too, during the period from December to July.

The results from both series of inoculation experiments are given in the above table.

From these results it is clear that of the five strains isolated from mound scab, one only. Strain 4, was pathogenic. Where used under the conditions of soil inoculation this strain produced scabs which were unmistakably identical with the mound scab from which it was derived. A photograph of one of the infected roots from Pot 7 is given in Plate XX. fig. 3. In the mixed inoculations the same strain in combination with Strain 1 and with Strain 2 also gave weak positive results but proved negative in combination with Strains 3 and 5. It is possible that at the time of these direct inoculations the roots were too old for infection to take place or that where it did occur the disease could not make headway. Strain 6, derived from the pitted scab, proved to be pathogenic in soil inoculations and also to a somewhat lesser extent in the direct inoculations. In the latter, only the Yellow Mangel was attacked, but in the former both Red and Yellow varieties showed a number of small dark pitted scabs closely resembling the original. Some of the older scabs consisted of cork tissue raised unevenly above the surface, similar to those which, as already mentioned, occur on the naturally infected mangel. Again, on one root there was a considerable amount of superficial scab on the upper part. This modification is easily accounted for



when the development of this type of scab has been studied (see later section on the histogeny of pitted scab) since here, owing perhaps to the relatively dry conditions, the parasite has been unable to penetrate the first layer of cork laid down and the tissue above has dried off.

A further and more important result arising from the soil inoculations made with Strain 6 was that the true terminal and lateral roots were also severely attacked by the organism. This attack showed itself in the form of numerous irregular, dark brown nodules of scab tissue scattered over the surface. The fine rootlets were similarly attacked for the most part at their bases where the nodules coalesced to form a slightly raised mass extending along the two longitudinal rows. This infection of the roots of mangel plants has not so far as we are aware been previously recorded and is of special interest in view of a similar infection which has been shown by Millard and Burr<sup>(1)</sup> to occur in the potato plant. A photograph of one of the affected roots from Pot II, Series 1, is shown in Plate XX, fig. 4. Lastly, from Series 2, Group 12, the interesting result was obtained that the potato scabbing organism *A. scabies* (Thaxter) Güssow, emend. M. and B., was also pathogenic and produced scabs identical in appearance with those of Strain 6. The *Actinomyces* Strains 4 and 6 were easily re-isolated from the artificially produced raised and pitted scabs respectively and are therefore regarded as the causative organisms of these two types.

#### *Definition of the Actinomyces Strains 4 and 6.*

The following media were selected for culturing these two organisms, the pH being indicated in brackets: saccharose synthetic agar (6·8), glycerine synthetic agar (6·8), dextrose synthetic agar (6·8), calcium malate glycerine agar (7·0), dextrose (asparagin) agar (Krainsky) (6·8), nutrient potato agar (7·0), gelatin (to 7·4), egg albumen agar (7·0), potato plug, mangel plug, tyrosin agar (6·9), starch nitrate agar, saccharose synthetic solution (6·8), glycerine synthetic solution (6·8), glucose broth (7·1), brom-cresol milk (7·0). With the exception of gelatin (grown at room temperature) the cultures were incubated at a temperature of 24° C. for 4 weeks. In addition to the above cultures nitrate reduction and starch hydrolysis tests were carried out and also tests for growth under anerobic conditions and at a temperature of 37° C. The methods adopted in sub-culture and in recording cultural changes were those recommended by Millard and Burr<sup>(4)</sup> and comparison with the species defined by them was thus easily possible. Strain 6 proved to be identical with *A. scabies* (Thaxter) Güssow, emend. M. and B., thus

proving an identity between potato and mangel scab in respect of the pitted type of this disease. It is interesting to recall that the organism in question is that which produces root nodules on potatoes as already mentioned. The external effects produced by it on its respective hosts are therefore almost identical. No parallel could be found between Strain 4 and any recorded species and since also the cultural characteristics of this strain are very definite it has been given specific rank with the name of *Actinomyces tumuli*. A description of the more characteristic cultures of this species are given at the end of this paper, and a photograph of a few of these is shown in Plate XXI, fig. 5.

#### THE STRUCTURE OF RAISED AND PITTED MANGEL SCABS.

An account is first given of the external features of these two types of scab.

##### (1) *Raised Scab.*

This consists of a mass of soft tissue 5-20 mm. in diameter rising 2-6 mm. from the surface of the mangel. The sides of the scab are fairly smooth but the upper surface may be roughened with small black patches of skin-like tissue which easily flake off. In some of the scabs the sides rise at right angles to the mangel surface and may even be waisted in the manner of a door handle; the surface is then flat or concave. In others, the scab forms a mound with gently sloping sides and the surface is convex. It seems possible then that raised scab should be subdivided into two types which we may call knob and mound scab, and detailed work on their mode of development and internal structure lends support to this view. If it be correct, then an interesting analogy is provided between the two types of raised scab arising on the mangel and those recorded by Millard and Burr<sup>(1)</sup> on the potato and ascribed by them to infection by different species of *Actinomyces*. In both mound and knob scabs a depression in the centre of the scab marks the point of infection.

##### (2) *Pitted Scab.*

This closely resembles potato scab of the same type and needs no further description than that given in the earlier part of this paper.

#### HISTOGENY OF MANGEL SCAB.

The minute anatomy of mangel scab is so dependent on the peculiar mode of growth of the mangel root that it seems advisable first to give an account of this as described by Eames and MacDaniels<sup>(1)</sup>.

"The first cambium of the seedling forms a ring of bundles close about the primary xylem. A secondary cambium soon arises in the pericycle and this is followed in rapid succession by others arising similarly. All layers continue to function perhaps indefinitely, though more slowly after an early period of activity. The cambium apparently arises as a continuous band, but soon forms more or less separate bundles, bands of conjunctive parenchyma developing between the vascular strips. The position of each new cambium as it arises in the pericycle is such that it encloses a few layers of pericyclic cells. These rapidly multiply and build up a parenchymatous layer. Alternate bands of proliferated pericycle and of vascular bundles are thus formed. The former constitute the dark coloured and the latter the light coloured rings in the beetroot. The bundles are themselves largely parenchymatous, but a few lignified cells occur in the xylem. Growth continues through all layers, in the bundles apparently both by cambium activity and by proliferation of the parenchyma of the xylem and phloem."

Again, discussing the function of the pericycle in roots on page 231, "The pericycle is commonly a persistent structure in roots even where secondary thickening is well developed. In such cases new cells are added by the division of the existing parenchyma so that the layer is not ruptured by the growth beneath it." "Its component cells are largely parenchymatous in their nature, and although they become permanent tissue early in the development of the root, they retain their ability to initiate new structures by the formation of secondary meristems." "It is in the pericycle that...in the majority of cases the first periderm layer is formed."

In regard to the formation of scab it will be seen that it is the capacity for proliferation of the parenchymatous cells of the pericycle and the possibility for a periderm layer to arise in this tissue which are of importance. It may be added that in a healthy mangel root the rind is composed of a cork layer from one to two cells thick.

#### *Mound Scab.*

Hand and microtome sections were first made of young scabs about 5 mm. in diameter. The sections were stained either with safranin and Delafield's haematoxylin or with Sudan III, and a microphotograph of one of these is shown in Plate XXII, fig. 6. The scab tissue consists almost entirely of a thick layer of degenerate and heavily suberised cortical tissue in which large cavities occur. Below this the outermost layers of the pericycle show obvious signs of the beginning of meristematic proliferation by the appearance of cross walls in the cells. No cork or cork cambium is as yet present. It is here indeed that the mangel differs so markedly from the potato. Priestley and Bebbington<sup>(5)</sup> find that whereas, in a cut potato a cork meristem arises under favourable conditions in a few days, the mangel and beet take from 3 to 5 weeks. A similar difference is found between these plants in the formation of cork in response to *Actinomyces* attack.

Older scabs of 10 mm. diameter were next sectioned and a photomicrograph of one of these is shown in Plate XXII, fig. 7. Here considerable proliferation of the pericyclic zone has taken place, which is greatest immediately below the seat of infection and decreases in proportion to the distance from this point. Consequently, the scab tissues take on an appearance of a mound with gently sloping sides. A cork layer has now been formed in the outermost layer of the pericycle, and as might be expected this is thickest at the central point of the scab where it shows 5-6 cells, and thinnest on the slopes where it shows only 1-2 cells. Above this cork there is, as before, a thick layer of degenerate and heavily suberised tissue which easily flakes off thus exposing a smooth cork layer. Lutman and Johnson<sup>(3)</sup> suggest that this easily removable dead tissue consists of a thickened layer of cork but this is entirely in disagreement with our experience and is indeed very improbable on physiological grounds. The small black plates often seen on the surface of these scabs consist of fragments of this debris still adhering to the cork. It may be noted that the bundles in the outer vascular ring below the scab are poorly developed.

#### *Knob Scab.*

Here it seems that cork formation takes place at a somewhat earlier stage in the development of the scab and is moreover greater. Plate XXII, fig. 8, shows a section through the centre of a knob scab where a plate of cork 6-8 cells thick has been formed immediately below the seat of infection. As in mound scab, increase in thickness of the pericycle ceases beneath the cork layer as soon as this has been formed, since further pressure from below would naturally tend to rupture it. Apparently, however, in knob scab even when cork has been formed, the irritation stimulus of the parasite is not exhausted and the pericyclic zone continues to proliferate with the result that there is a rapid upward and outward growth of the tissues on either side of the central cork plate. Thus the upper surface of the scab tends to become concave and the bulge of tissue to develop a waist. The pressure of this growth is often sufficiently great to rupture the normal surface cork layer. Eventually, a cork layer 1-2 cells thick arises in the outermost layers of this new pericyclic tissue along the steep slopes of the scab. If, as seems probable, the mound and knob scabs are caused by two distinct strains of *Actinomyces*, then there is little doubt that that causing the latter exerts the stronger irritation stimulus. A common feature of the invading organism in both scabs is that it is unable to penetrate deeply into the tissues

but having established itself spreads laterally through the cortical tissues.

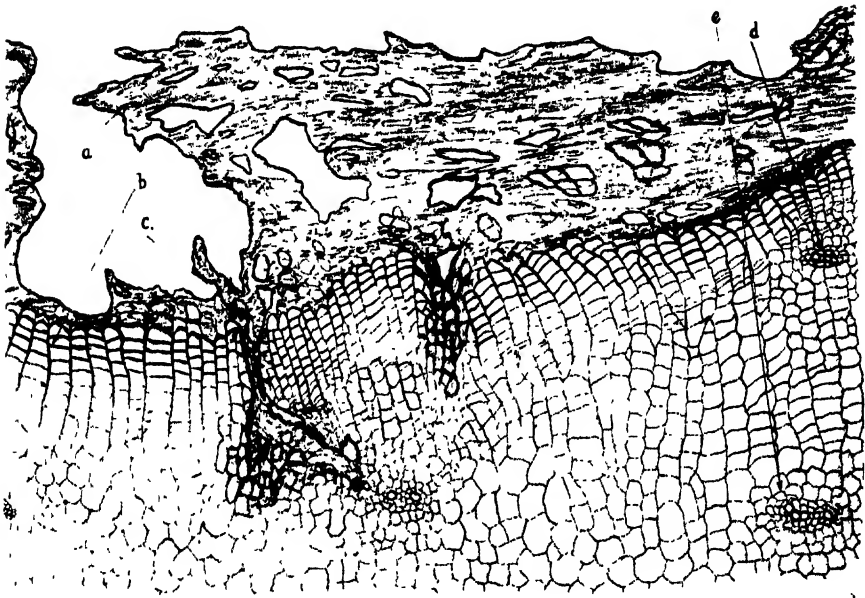
A further interesting feature of knob scab is that one or more new vascular rings form in the proliferated pericycle of the scab tissue as seen in Plate XXII, figs. 8 and 9. The vascular elements in these adventitious rings are often, however, poorly developed. Lutman and Johnson (3) state that in raised scabs on garden beet "the protruding portion is due to an excess in growth of the rings directly under the infected spot," but in the field mangels with which we have worked we have not found this to be the case. Indeed, it seems clear from the Fig. No. 2 given by these workers that the protruding portion of the scab shown has originated by proliferation of the pericycle. It must be remembered, that at the time this work was published the view of De Bary was generally accepted that each zone of tissue, whether vascular or parenchymatous, in the growing root originated entirely from the cambium of a vascular ring and this probably influenced the authors in the interpretation of their results. Again, whilst in the raised scabs we have examined, the scab tissues are definitely pericyclic in origin, a certain slight bowing out of the normal vascular rings in the direction of the scab does occur and it is not impossible that the new rings laid down in this scab tissue may link up with the former giving a fallacious appearance of common origin.

#### *Pitted Scab.*

This form of scab is radically different in its development from the raised types and approximates much more closely to the pitted scabs of potatoes. A section is shown in Text-fig. 1. The invading organism ramifies in the cortical parenchyma and then penetrates deeply into the tissues easily passing through the outer vascular ring. It reduces these tissues to a vacuolated pulpy mass. In the meantime, no proliferation of the pericycle takes place as in mound or knob scab but, on the other hand, a very active cork meristem arises in the pericycle from which a layer of cork up to 10 cells thick is ultimately formed. In the illustration given, this cork layer cuts across the outer vascular ring and it is thus clear that it is not formed from the normal cambium as suggested by Lutman and Johnson (3). Even this thick layer of cork does not suffice to check the organism which probably passes through it before suberisation can take place and frequently penetrates to the second ring of vascular bundles or even further.

It is thus obvious that the tissue destruction caused by pitted scab

is much greater than that of the more noticeable raised scabs. In older scabs of this type the scabs become slightly elevated above the surface by the upward pressure of successive cork layers arising below the first.



Text-fig. 1. Radial section through a pitted scab—drawn under the camera lucida. Magnification  $\times 95$ . (a) Disorganised and vacuolated tissues of the cortex and outer pericycle. (b) Cork layer (the outer cells of which are strongly suberised) passing from the outer pericycle at the edges of the scab through the first vascular ring into the second pericycle ring at the centre of the scab. (c) Penetration of the organism through the cork layer. (d) One bundle of the outer vascular ring. (e) Second vascular ring—so far, intact.

#### SUMMARY.

It is shown that scab of mangels is of two distinct types—raised and pitted. The raised type may be further sub-divided into two which are called mound and knob scab respectively. These latter types appear to develop more commonly on yellow-skinned varieties.

Investigation of the development and structure of these scabs reveals a striking difference in their origin. In mound and knob scab the first response of the host to parasitic attack is an active meristematic proliferation of the outer pericycle which forms a mound-like protuberance. Some time afterwards—not less than a month—a cork meristem arises in the outer layers of this new pericyclic tissue and the mound becomes

capped with a layer of cork several cells thick at the apex and very thin over the slopes. In mound scab no further growth seems to occur but in knob scab the pericycle continues to proliferate and the new tissue, prevented from growing directly upwards, gives rise to the overhanging bulges so characteristic of this type. For the same reason the surface of this type of scab tends to become concave.

It is thus shown that raised mangel scabs are not formed from the cambium of the vascular rings. On the other hand, the normal vascular rings may bow out slightly towards the scab tissues and one or more adventitious rings of vascular bundles frequently arise in the proliferated pericycle of the scab tissue.

In both types of raised scab the outer dead tissues easily flake off thus exposing a smooth corky surface, but portions often remain adhering to this in small black patches. The point of infection in both scabs is marked by a central depression in the wart.

In pitted scab no proliferation of the pericycle takes place and thus, at first, a shallow pit is formed. A cork meristem then arises in the outer pericycle giving rise to a saucer-shaped cork layer up to 10 cells thick. This is frequently penetrated by the organism which may reach and destroy the first and possibly the second vascular rings at the seat of infection. Further and more deeply seated layers of cork then arise and the scab tissues are thereby forced upwards forming a wrinkled, corky scab slightly raised above the surface. Pitted scab formation in the mangel is thus very similar to that of the ordinary type of common scab in potatoes.

From mound scab a strain of *Actinomyces* has been isolated which reproduced the same type in inoculation experiments. The more outstanding cultural characteristics of this strain have been described and since it is new to the literature and appears to merit specific rank it has been named *Actinomyces tumuli*.

From pitted scab a strain of *Actinomyces* was isolated which also reproduced its own type in inoculation experiments. In addition, in these experiments the organism attacked the true roots and fibrous rootlets of the inoculated mangel plants on which it produced numerous characteristic, dark brown, nodular outgrowths.

This organism proved to be identical with *A. scabies* (Thaxter) Güssow, emend. M. and B. The same type of scab was produced in inoculation experiments with a stock culture of this organism.

*Description of the more characteristic cultures of Actinomyces*  
*Strain 4—A. tumuli.*

The cultures were examined at weekly intervals and the descriptions given below record the more outstanding changes which occurred during four weeks. The numbers used to indicate these changes often approximate to, but do not accurately stand for weekly changes.

Colours given in inverted commas are taken from Ridgway's *Color Standards and Color Nomenclature*.

SOLID MEDIA.

SACCHAROSE SYNTHETIC AGAR.

*Colonies.* Small, with bowl-like projections into medium.

*Growth.* Fair, "Pale Smoke Grey" becoming darkly opaque.

*Aerial mycelium.* Arises on centre of colony; later, general. (1) White; (2) "Pallid Neutral Grey."

*Guttation.* Colourless drops leaving small black craters.

*Pigment.* None.

GLYCERINE SYNTHETIC AGAR. Plate XXI, fig. 5 (1).

*Colonies.* Raised centres with wide flat rims; round, forming hemispherical growth in medium.

*Growth.* Fair; later, good.

*Aerial mycelium.* Scant at first, borne centrally; later, general; white.

*Guttation.* At first numerous small colourless drops; later, coalescing to form sector-shaped drops; leaving a circle of black craters giving the colony a wheel-like appearance.

*Pigment.* (1) "Ecrú Olive"; (2) "Tawny Olive."

DEXTROSE SYNTHETIC AGAR. Plate XXI, fig. 5 (2).

*Colonies.* Large round, with hemispherical growth in medium. The centres of the colonies become wrinkled and a flat margin develops.

*Growth.* Good, "Pale Smoke Grey."

*Aerial mycelium.* Arises in concentric rings round a dark bare centre; later, general, on margin only. White.

*Guttation.* None.

*Pigment.* (1) "Buffy Olive"; (2) "Tawny Olive."

CALCIUM MALATE GLYCERINE AGAR. Plate XXI, fig. 5 (3).

*Colonies.* Finely fringed; hemispherical growth in medium.

*Growth.* Fair; later good. "Pale Smoke Grey."

*Aerial mycelium.* Annular at first; white; later, general, "Pale Smoke Grey."

*Guttation.* A ring of small droplets on each colony, leaving ring of craters.

*Pigment.* Faint brownish.



**DEXTROSE ASPARAGIN AGAR (Krainsky). Plate XXI, fig. 5 (4).**

*Colonies.* Raised, with bowl-like growth in medium, margins finely fringed.

*Growth.* Fair, later, good. "Pale Smoke Grey."

*Aerial mycelium.* (1) On fringe only, white; (2) general, "Pallid Neutral Grey."

*Guttation.* Arising after second week; minute colourless drops leaving minute craters.

*Pigment.* Slight. "Ecu Olive."

**NUTRIENT POTATO AGAR. Plate XXI, fig. 5 (5).**

*Colonies.* Umbonate with broad flat margin and bowl-like growth in medium.

*Growth.* Good, lustrous, slimy. "Pale Smoke Grey."

*Aerial mycelium.* None.

*Guttation.* None.

*Pigment.* None.

**GELATINE.**

*Growth.* Beaded with clump at base of liquefied basin.

*Aerial mycelium.* None.

*Liquefaction.* Stratiform, complete in two weeks, followed by digestion and production of clear liquid.

*Pigment.* None.

**EGG ALBUMEN AGAR.**

*Growth.* Good, ramifying into medium.

*Aerial mycelium.* Arises in centres of colonies, white; later, general, dull white to "Pale Smoke Grey."

*Guttation.* Small colourless drops leaving dark craters.

*Pigment.* None.

**POTATO PLUG. Plate XXI, fig. 5 (6).**

*Growth.* Heavy, slimy, puckered, black. Later, a flat margin of "Greyish Olive" growth appears.

*Aerial mycelium.* None.

*Colour of plug.* Greyish brown.

**MANGEL PLUG.**

*Growth.* Good, pale, translucent, raised.

*Aerial mycelium.* At first, scanty, white; later, abundant, "Pallid Mouse Grey."

**TYROSIN REACTION. Negative.****GROWTH UNDER ANAEROBIC CONDITIONS. None.****STARCH PLATE. Positive. Width of hydrolytic zone complete 10 mm., incomplete 15 mm.****NITRATE REDUCTION. Positive. Width of colour zone, 20 mm.****GROWTH AT 37° C. None.****LIQUID MEDIA.****SACCHAROSE SYNTHETIC SOLUTION.**

*Growth.* Fair, star-shaped colonies at base and on sides of tube.

*Surface growth.* None.

*Pigment.* None.



Fig. 2

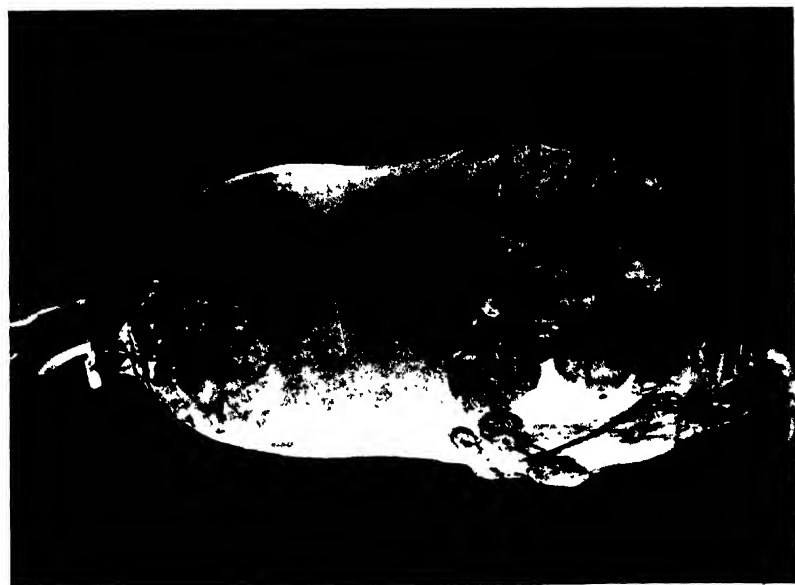


Fig. 1.



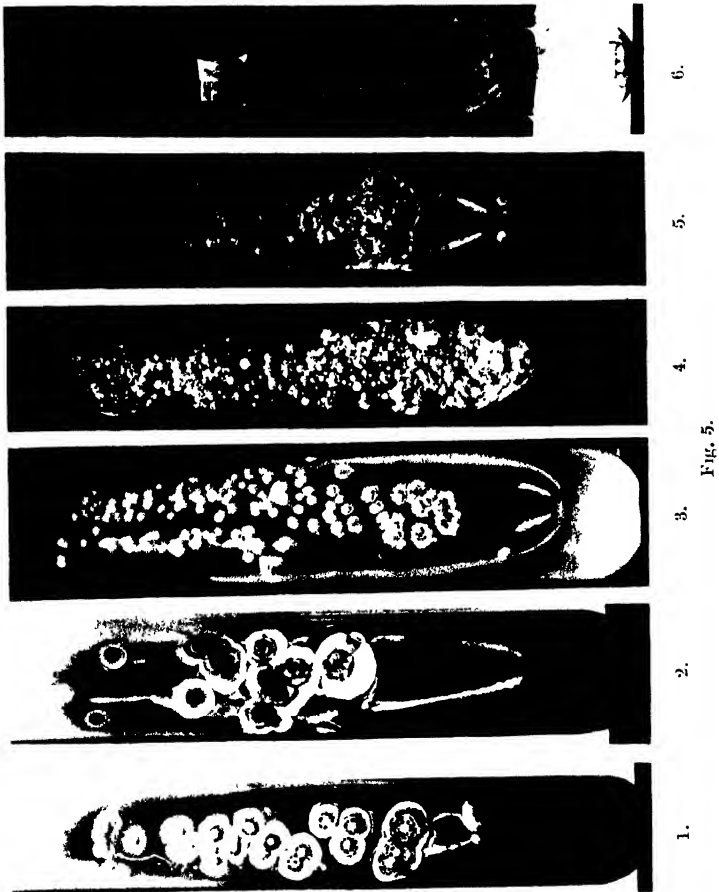


Fig. 4



Fig. 3





MILLARD & BEELEY MANGEL SCAR—ITS CAUSE AND HISTOGENY (pp. 296-311).



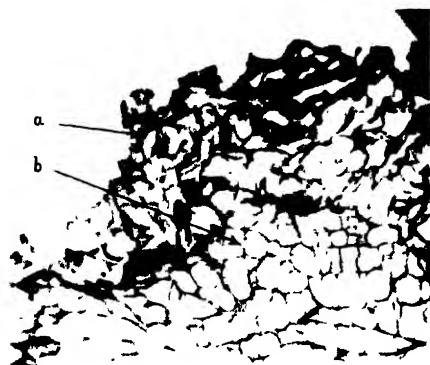


Fig. 6.



Fig. 8

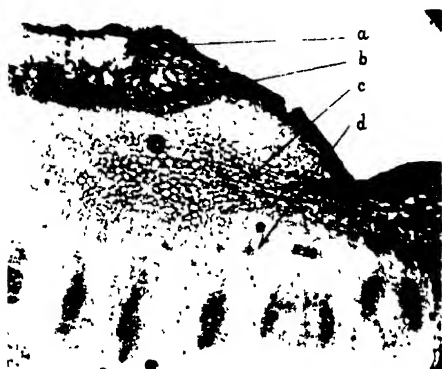


Fig. 7.



Fig. 9.





## GLYCERINE SYNTHETIC SOLUTION.

*Growth.* Fair, dense star-shaped colonies at base and on sides of tube.

*Surface growth.* None.

*Pigment.* None.

## GLUCOSE BROTH.

*Growth.* Fair, large white spherical colonies at base.

*Surface growth.* None.

*Pigment.* None.

## BROM-CRESOL MILK.

*Growth.* Fair; later, good.

*Aerial mycelium.* None.

*Coagulation.* Definite clotting. Colour of clot "Water Green."

*Digestion of clot.* Slight.

*Colour of medium.* (1) "Court Grey"; (2) "Water Green."

## REFERENCES.

- (1) EAMES and MACDANIELS (1925). *Introduction to Plant Anatomy Text-Book*. McGraw-Hill Book Co.
- (2) KRUGER, F. (1904). Untersuchungen über den Gurtelschorf der Zuckerrüben. *Arb. Biol. Anst.* IV, 3.
- (3) LUTMAN and JOHNSON (1915). *Phytopathology*, v.
- (4) MILLARD and BURR (1926). *Ann. Appl. Biol.* XIII, No. 4.
- (5) PRIESTLEY and BEBBINGTON, *loc. cit.*
- (6) WOLLENWEBER, H. W. (1920). *Der Kartoffelschorf*, Heft 2. Arbeiten des Forschungsinstitutes für Kartoffelbau.

## EXPLANATION OF PLATES XIX—XXII.

- Fig. 1. Raised scab mainly of knob type (natural infection) on Golden Tankard mangel.
- Fig. 2. Raised and pitted scabs (natural infection) on Red Intermediate mangel. (a) Young pitted scabs.
- Fig. 3. Mound scab on Yellow Globe mangel produced by inoculation with *Actinomyces* Strain 4 (Series I, Pot 7). The scab on the right has been scooped out for inoculum in reisolating the organism.
- Fig. 4. Root infection produced on Red Intermediate mangel by inoculation with *Actinomyces* Strain 6 (Series I, Pot 11). Note also the swollen tissues around the bases of the rootlets on the "bulb" caused by coalescence of the scab nodules.
- Fig. 5. Cultures of *Actinomyces tumuli* on (1) glycerine synthetic agar, (2) dextrose synthetic agar, (3) calcium malate glycerine agar, (4) dextrose asparagin agar, (5) nutrient potato agar, (6) potato plug.
- Fig. 6. Radial section through a young mound scab showing (a) disorganised cortical tissue, (b) meristematic activity commencing in the outer layers of the pericycle.
- Fig. 7. Radial section through an old mound scab showing (a) disorganised cortical tissue, (b) cork layer heavily suberised, (c) proliferated pericycle forming scab tissue, (d) outer vascular ring of the root in which the vascular elements are poorly developed.
- Fig. 8. Radial section through centre of a knob scab showing (a) disorganised cortical tissue, (b) cork layer heavily suberised underlying the affected area, (c) proliferated pericycle, (d) lateral and upward outgrowth formed by the proliferating pericycle, (e) adventitious vascular ring formed in the scab tissue.
- Fig. 9. Radial section through outer part of knob scab showing waist. (a) Disorganised cortical tissue, (b) proliferated pericycle on the outer edge of which is a cork layer 1-2 cells thick, (c) adventitious vascular rings formed in the scab tissue.

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# A STUDY OF *HYLEMYIA* (*CHORTOPHILA*) *BRASSICAE* BOUCHÉ, THE CABBAGE ROOT FLY AND ITS PARASITES. WITH NOTES ON SOME OTHER DIPTEROUS PESTS OF CRUCIFEROUS PLANTS

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(With Plate XXIII and 10 Text-figures.)

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## INTRODUCTION.

ALTHOUGH much attention has been paid in Canada and the U.S.A. to the study of the bionomics of *Hylemyia brassicae*, the cabbage root fly, it has been almost entirely neglected by the entomologists of this country and in spite of its long-continued and injurious depredations no detailed account of the fly in the British Isles exists. The writer therefore presents

this study of the cabbage root fly and its parasites in the hope that it will fill a gap in the knowledge of the injurious root-feeding insects of the country.

The writer is indebted to Mr H. Britten, Mr J. E. Collin, Mr G. T. Lyle and Dr J. Waterston for their kind assistance in the identification of many of the insects concerned in this study, to Mr James Wood for collecting material and to Miss Maud Jepson for her help in preparing the text-figures.

#### SYNONYMY.

Bezzi and Stein(7) refer to this fly as *Chortophila brassicae* Bouché, giving as synonyms *Anthomyia appendiculata* Big., *A. floccosa* Macq., *A. floralis* Fall. and *A. villipes* Zett. In 1843 Curtis(3) described an insect which he bred from cabbage roots as *Anthomyia radicum* which was probably *H. brassicae*. Gordon Hewitt(6) however describes an insect under this name which is obviously different from that described by Curtis. Seguy (11), pp. 107-8) also gives a description of *Anthomyia radicum* under the genus *Chortophila* sub-genus *Paregle*. Slingerland(12) gives a detailed historical account of the synonymy of *H. brassicae*. He, like other American and Canadian writers, refers to this insect as *Phorbia brassicae*. *Phorbia* being synonymous with our genus *Chortophila*. In 1923 Seguy(11) placed this fly in the genus *Hylemyia*, calling it *Hylemyia brassicae* Bouché. This would appear to be the correct genus as *Hylemyia antiqua*, the onion fly, a very closely allied form, has long been placed in the same genus. To be consistent therefore with an earlier paper(13) the writer has referred to the cabbage root fly throughout this work as *Hylemyia brassicae* Bouché.

#### METHOD OF STUDY.

The work was carried out in Lancashire and Cheshire, and the insect was studied in the field, insectary and laboratory. After some unsuccessful attempts the fly was induced to breed in captivity under the following conditions: cabbage and cauliflower plants were grown in 12-inch pots, over each was placed a glass cylinder, 2½ ft. long with a diameter of 10 in., the top of the cylinder being closed with a piece of muslin. The insects were put into these breeding cages and were fed upon sugar and water. Under these conditions it was possible to breed a continuous series of flies throughout the season, and good opportunity was thus afforded for observing times of emergence, duration of the various periods, number of generations, etc.

Much detailed observation was also made of the insect in the field upon the cruciferous crops of the counties and upon a large plot of cabbages and cauliflowers grown for this purpose.

#### DESCRIPTION AND DURATION OF STAGES.

##### *The Egg.*

The egg is white and easily visible. In shape it is cylindrical, one end being pointed, the other somewhat truncated: in length it measures about 1 mm. and is less than half that in width. The shell or chorion is sculptured into longitudinal ridges; down one side of the egg for three-quarters of its length run two well-marked parallel sutures. Between these sutures is a somewhat depressed area in which the ridged sculpturing is less pronounced than that in the rest of the chorion. When the egg hatches, the smoother area between the sutures is pushed out, the larva emerging at the truncated end where the space between the sutures is widest.

*Duration of Egg Stage.* From a number of observations in field and laboratory, it would appear that from four to five days is the normal duration of the egg stage. A fly was observed ovipositing on young cabbage plants in the field on May 31st. After some half dozen eggs had been laid, they were collected and placed under observation in the laboratory. They hatched in the early hours of the morning of June 4th. Similar observations a week later also gave an average duration of the egg period as four days. This length of time however may vary as in some cases, especially the second generation, the length of the egg period was reduced to three days.

##### *The Larva.*

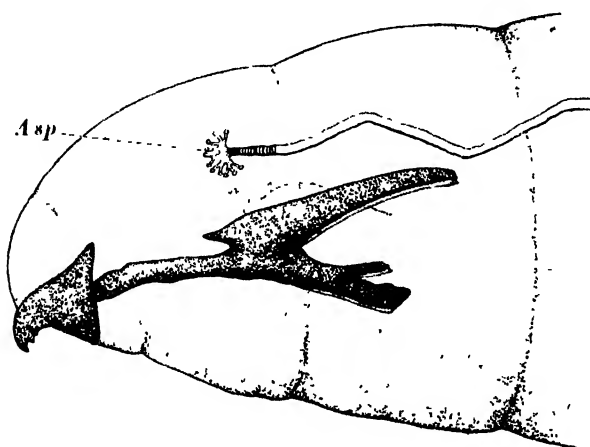
*First Instar.* On hatching from the egg the young maggot measures slightly over 1 mm. in length. It is curved, white in colour and the segments and tubercles are distinct. In this stage there are no anterior spiracles and the buccal skeleton differs slightly in appearance from that of the second and third instars. Text-fig. 1 is a drawing of the first-stage larva.

*Second Instar.* The second-stage larva measures from 2 to 4 mm. in length. Both anterior and posterior spiracles are now present but the posterior spiracles differ from those of the third instar in having only two openings in each spiracle instead of three. *Vide* Text-fig. 4 B.

*Third Instar.* The third-stage larva varies from 2 to 8 mm. in length and from 1 to 2 mm. in diameter. It is white, tapering at one end and



Fig. 1. First-stage larva of *H. brassicae*.



A.s.p. = anterior spiracles.

Fig. 2. Anterior segments of mature larva of *H. brassicae*.

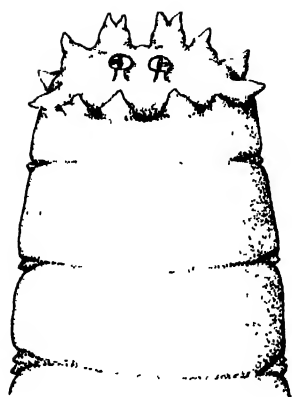


Fig. 3. Posterior segments of mature larva showing arrangement of tubercles.

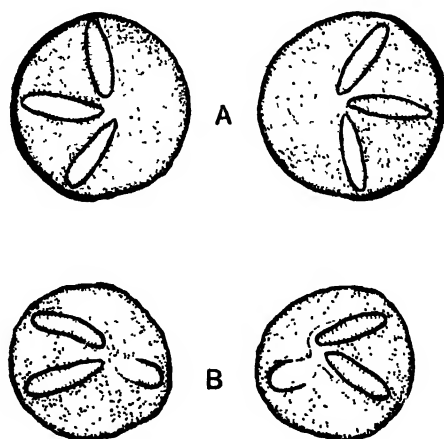


Fig. 4. Posterior spiracles. A, mature larva. B, second-stage larva. In B the third spiracular opening is in process of being formed.

truncated at the other, the caudal end. The integument is somewhat smooth and shining; this fact constitutes a point of difference between it and some other maggots of similar habits. At the anterior end there is a strong buccal skeleton, the lateral plate being forked, *vide* Text-fig. 2. The anterior spiracles consist of a pair of fan-shaped organs situated laterally containing from 11 to 13 processes. The number of these processes is not constant even in individual larvae, occasionally 11 being present on one side and 13 on the other. The number of these processes varies between 10 and 13 in the mature larva and possibly rather fewer in the second-stage larva. There are two pairs of tubercles surrounding the buccal opening. At the posterior end there is a caudal corona consisting of six pairs of tubercles, while a seventh pair is situated ventrally near the anus. The outstanding feature of this corona is the pair of bifid or forked tubercles on the ventral rim of the last segment, these forked tubercles are important in the identification of this larva, cf. Figs. 7-10. The arrangement of the caudal tubercles is illustrated in Text-fig. 3. In the centre of the caudal disc arise the knob-like posterior spiracles which are the external openings of the tracheae, they are brownish yellow in colour, heavily chitinised and each one opens with three slits. Text-fig. 4 is a drawing of these posterior spiracles showing the slit-like openings.

*Duration of Larval Life.* In 12 series of larvae of the first and second generation under observation in the insectary, for the duration of the larval stage, the periods varied between 19 and 25 days with an average of 23 days. Observations on the earlier maggots of the third generation gave an approximately similar period but a considerable proportion of the later maggots of this generation took much longer to pupate, some existing in the larval condition for as long as 35 days.

From observations made the writer considers it possible that some larvae of the third generation may hibernate as such. A number of maggots hatching from a batch of eggs on September 4th fed normally till the 21st of the month, being by that time nearly full grown. A spell of cold weather then set in and the maggots ceased feeding and appeared to rest in a state of suspended animation. One larva was removed to the greater warmth of the laboratory where it immediately pupated, a week later a second was removed under similar conditions with the same result. Meanwhile of the remaining larvae, each one had hollowed out a small cell in the cabbage root where it remained till the end of the first week in October, when the temperature rose and the larvae commenced to pupate, the last one transforming on October 11th. It is of

interest to note that examination of these quiescent larvae showed them to be of unusual size and to be provided with a large quantity of fat as if in preparation for a long rest. It is thus possible that the maggots found in cabbage roots during November and December are hibernating, although all maggots in the insectary had pupated by October 25th.

### *The Puparium.*

The puparium is a seed-like object rounded at both ends. It is very variable both in size and colour, the more normal specimen measuring 6-7 mm. long by 3 mm. broad at the centre and being of a reddish brown colour. Often however puparia are found of approximately half this measurement, the variation in size being probably governed by the amount of food available for the larva. The colour also varies from deep red to light brown. The integument of the puparium consists of the hardened skin of the larva, and some larval characteristics are still visible in the shape of the bifid tubercles at the posterior end of the puparium and the spiracles at the anterior end. Text-fig. 5 is a drawing of the puparium.

*Duration of the Pupal Stage.* That the length of the pupal period is very variable is shown by the following observations. Out of a total of 51 first-generation pupae, 29 gave an average duration of 15 days, the remaining 22 gave an average period of 35 days, the longest time of this generation being from June 29th to September 6th, *i.e.* 69 days, and the shortest period from July 8th to July 19th, *i.e.* 11 days. From a similar series of observations on the second-generation pupae the average period was 15 days, the longest period being 18 days and the shortest 7 days. A considerable proportion of this brood did not hatch



Fig. 5. Puparium of *H. brassicae*.  
B.T. Bifid tubercles of larva.



until the following year. The third-generation pupae hibernated *in toto*, giving rise to adults in the following spring.

### *The Adult.*

*Male Fly.* The size is variable, averaging 6 mm. It is dark grey in colour with lighter markings. The eyes are sub-contiguous and occupy the greater portion of the head. Dorsally the thorax possesses three well-marked longitudinal bands, the centre band being continued over the abdomen which is rather small and tapering. The squamae are large, wing veins brown. The legs are black with a cluster of bristles on the hind femora, while the whole fly is thickly covered with black bristles.

*Female Fly.* Eyes widely separated, body much lighter in colour than the foregoing, abdomen broader and more pointed, legs brownish grey without the tuft of bristles characteristic of the male. As a whole the female fly is less bristly.

*Duration of Life of Adult.* When fed on sugar and water the flies lived in captivity for considerable periods; both sexes lived for as long as 35 days, and in one or two cases even longer. Duration of life in the male ranged from 9 to 35 days and in the female 14 to 38 days. If no food was provided the male flies commenced to die after 3 days and the females after 4 or 5 days. It may be of some interest to note in this connection the extraordinary degree to which the flies in captivity, especially the females, would gorge themselves upon the sugar and water supplied to them. The writer had under observation two females which had become enlarged by this procedure to almost twice their normal size. The abdomen was distended until it was nearly circular, the proboscis was extended and could not be withdrawn, and the antennae were fixed in an abnormal upright position giving a horned appearance to the insects. These two flies were removed, placed in an empty glass vessel, and kept under observation. After 2 or 3 days the swelling began to subside, until, a week after their transfer to the empty jar, the flies had resumed their normal appearance, apparently little the worse for their adventure. This desire for sugar might be profitably employed in the use of a poison bait on the lines of that so often recommended for the onion fly.

*Total Length of Life-cycle.* From observations made in the insectary upon flies bred in captivity, the average length of the life-cycle from egg to adult would appear to be 46 days, with a minimum observed period of 37 days and a maximum of 53 days.

## LIFE-HISTORY AND HABITS.

*Number of Generations.*

There are three generations of *H. brassicae* in the year. By the method of continuous breeding adopted in this study, it was possible to observe the number of broods per season with considerable accuracy. As in the case of *H. antiqua*, the onion fly, a closely allied insect, there is no sharp line of demarcation between each brood, and larvae of the second generation were often still feeding at the same time as larvae of the third brood. Similarly some second-generation adults were still hatching during the period of maximum emergence of the third-generation adults. The flies of the first generation hatching from the overwintering puparia begin to appear about the end of the second week in May, the earliest record for this district being May 14th.

In the present year (1926) females were first observed ovipositing on May 31st. The first fly of the second generation hatched in the insectary on June 29th, the time of maximum emergence being from July 8th to July 18th, although flies of the second brood continued to emerge at infrequent intervals throughout August, the last to emerge appearing on September 6th, after having pupated on June 29th. The first adults of the third generation commenced hatching on July 27th, the period of maximum emergence being from August 7th to 13th. The third generation continued to emerge throughout August up to the second week in September, the last adult female observed being collected in the field on September 29th. This fly died almost immediately, all the eggs having been deposited as was revealed by an examination of the insect's oviducts. It is of interest to note that a considerable proportion of the second-generation pupae which normally should give rise to third-brood flies did not hatch at all in the year of pupation but remained in the pupal condition until the following year. This same phenomenon is to be found in the case of other root-feeding species.

*Reproduction.*

*Oviposition.* Prior to laying her eggs the female fly examines the soil surrounding the stalk of the cabbage or other cruciferous plant, prodding and testing the cracks with extended ovipositor. As a rule the eggs are deposited on the stem at, or just below, the soil level. Often however they are placed in the soil immediately surrounding the stalk or in the cavity between stalk and soil. The number of eggs deposited

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at one time varies from two or three up to a dozen or more; one female fly under observation deposited six before moving away to fresh plants. These remarks apply chiefly to the first two generations of flies; in con-

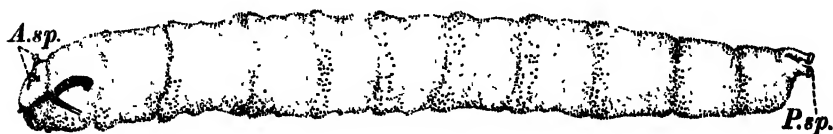


Fig. 6. Larva of *Phytomyza rufipes* Meig. *A.sp.*, anterior spiracles. *P.sp.*, posterior spiracles.

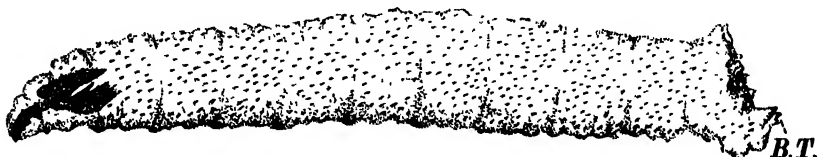


Fig. 7. Dipterous larva of unknown species from cabbage root. *B.T.*, one bifid tubercle.

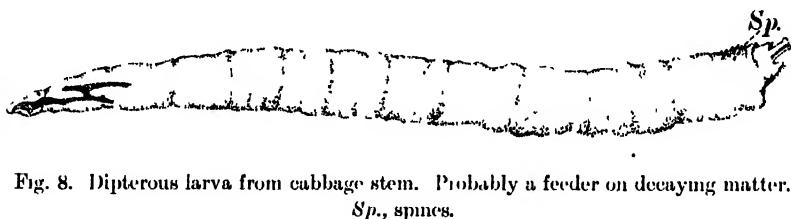


Fig. 8. Dipterous larva from cabbage stem. Probably a feeder on decaying matter. *Sp.*, spines.

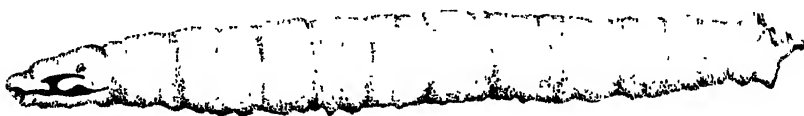


Fig. 9. Dipterous larva from cabbage stem. Probably a feeder on decaying matter.



Fig. 10. Larva of *H. brassicae* for comparison with Figs. 6-9. *A.sp.*, anterior spiracles. *P.sp.*, posterior spiracles. *B.Tt.*, two bifid tubercles.

sidering the egg-laying habits of the third generation and especially the latterly hatched flies of this brood, a somewhat different habit is revealed. On the three days September 15th-17th the writer collected

150 eggs from the following situations—deposited singly or in pairs at regular intervals along the midrib and larger veins of cauliflower and cabbage leaves, in fair numbers on the cut surface of cauliflower stems where the head had been removed, and also inside the cauliflower head, one or two eggs on each stalk of the inflorescence. These eggs were collected and kept under observation in the laboratory in order that their identity might be definitely established although external examination revealed no difference from the normal eggs of *H. brassicae*. Of these eggs a considerable proportion hatched into the cabbage-root maggot, there were also however larvae of different species of Anthomyids (*vide* Text-figs. 6–10), which latter had probably been induced to oviposit by the beginnings of decay in the plants in question. If further proof be needed, the presence of *H. brassicae* larvae in the midrib and large veins of cauliflower and cabbage leaves in late September seems indicative of a difference between the egg-laying habits of the third generation and of the two preceding it. The fact that in the autumn the stems of Brassicas are hard and woody is probably sufficient reason for a change in the place of egg deposition. Theobald (15) describes cases of *H. brassicae* larvae in the midribs of cauliflower and cabbage leaves and Gibson and Treherne (5), p. 24) also mention that they have observed the eggs of this fly deposited on the fleshy leaf stalks, though apparently they are of opinion that this is a rare occurrence.

*Pre-oviposition Period.* The following observations were made on the time elapsing between date of hatching and date of oviposition.

Adults emerged July 9th, eggs deposited July 18th			
„	„	10th,	„ 19th
„	Aug. 8th,	„	Aug. 15th
„	„ 15th.	„	„ 21st–22nd

Another batch of adults hatching on August 18th was noticed *in coitu* on August 26th, the eggs being deposited 24 hours later, *i.e.* August 27th. Females dissected 5 days after emergence were still immature but others dissected 8 days after hatching possessed some mature ova. From these observations the pre-oviposition period would appear to last between 7 and 8 days. Gibson and Treherne (5), p. 27) put the period at 6 days, and Schoene (10) from 3 to 5 days.

#### *Pupation.*

Pupation takes place in the soil surrounding the roots of the host plant, though occasionally puparia are found adhering to the root itself. In an examination in the field of a large number of infested plants the

majority of puparia were found at a depth of 3 to 5 in., while some, particularly of the later broods, were as deep as 8 or 9 in. Occasionally the larvae migrate laterally and puparia may thus be found near the surface of the soil 3 or 4 in. away from the plant. The adult insect seems able to emerge through a considerable depth of soil; in the laboratory pupae placed at the bottom of tall glass cylinders gave rise to flies which emerged easily through the 7 in. of soil superimposed.

### *Hibernation.*

The most usual method of hibernation is undoubtedly in the pupal stage. There is however certain evidence that the larvae may also hibernate. The writer has observed on several occasions large larvae with an unusual accumulation of fat in cabbage roots during October. These larvae did not appear to be feeding but remained motionless in small cells in the tissue. There is no doubt that a low temperature influences the larval development, and even if the larvae do not actually hibernate, they are capable of remaining in a state of suspended animation for considerable periods before finally pupating. Miles<sup>(9)</sup> records the larvae feeding in the heads of cauliflowers as late as November. These larvae had probably developed from eggs deposited in the heads of the cauliflowers by late-hatched flies of the third generation. Gibson and Treherne<sup>(5)</sup> and Schoene<sup>(10)</sup> also state that they have found larvae throughout the winter. It is thus reasonable to postulate that a small percentage of larvae do hibernate but in the writer's opinion they are capable of surviving only mild winters.

As regards hibernation in the adult form, evidence on this point is very scanty. Various writers have at different times put forward the view that the adult flies do survive the winter, but up to date no convincing evidence has been offered on this point. There is no doubt that the pupal stage is the usual condition for hibernation.

### *Feeding Habits of Larva and Injuries to Plants.*

On hatching from the egg the young larva as a rule makes for the root just below soil level, bores into the cortex and tunnels up and down. A plant is usually able by throwing out adventitious rootlets to withstand attack by a few maggots only, but where a dozen or more are present the whole root becomes hollowed out and the plant quickly dies. Although the name "root maggot" has been applied to the larvae of *H. brassicae*, the root is by no means the only, though possibly the most common, habitat of these maggots. The writer has had experience of a

field of cauliflowers which, although apparently otherwise healthy, yet were entirely blind. An examination of these plants revealed the presence of *H. brassicae* larvae in large numbers feeding at the apex of the stem and thus preventing the formation of the head. The roots however of these cauliflowers were quite healthy and untouched by the larvae. This habit of feeding in the heads of cauliflowers is more typical of the late brood, as is revealed by the oviposition habits of the third generation. The larvae have also been observed feeding in the growing points of turnips. In autumn, when the roots of cauliflowers and cabbages are hard and woody, the later larvae of the third brood may sometimes be found tunnelling in the midrib and large veins of *Brassica* leaves and also at the leaf bases. In October the writer has found the larvae to be more common in turnips than in Brassicas.

Injury to host plant depends therefore on the place of attack and number of maggots attacking. From an examination in the field of some 200 infested cabbage plants, the number of maggots in the roots varied from 9 to 46. Gibson and Treherne (5), p. 21) have found up to 127 maggots in a single plant, but such a large number is unusual in this country. The symptoms of maggot attack at the roots of Brassicas are characteristic. The plant first of all becomes limp and flaccid, the leaves turning bluish, later becoming yellow with complete collapse in a bad attack.

*H. brassicae* larvae may therefore be looked for in the following situations: (a) In the roots of Cruciferous plants, the most usual mode of attack. (b) In the growing point of turnips and of cauliflowers before the head is formed. (c) In the inflorescence of cauliflowers. (d) In the midrib and larger leaf veins of Brassicas. This last mode of attack should not be confused with that by another species of Dipterous maggot (*vide* Text-fig. 6) which normally tunnels in the midrib of *Brassica* leaves.

#### *Food Plants.*

The food plants of *H. brassicae* seem to be confined almost wholly to the Cruciferae. The larvae occur commonly in cabbage, cauliflower, brussels sprouts, kale, radish, turnips and swedes and the writer also has had experience of their feeding at the roots of stocks. Leaflet No. 122 of the Ministry of Agriculture gives several wild Cruciferous plants as hosts of this maggot, such as shepherd's purse, charlock, hedge mustard, etc. Chittenden(2) records celery as an occasional host plant and Gibson and Treherne (5), p. 20) give instances of damage to beet. As a rule however Cruciferous plants only are attacked. It is doubtful whether

there is much preference for attack within the varieties of Brassicas; the fly chooses young and tender plants of cabbage, cauliflower, or brussels sprouts apparently indiscriminately. A field of young sprout seedlings growing alongside a field of young cabbage was observed to be quite as heavily infested as the latter. Probably however, when the sprouts are more mature, infection is less common than with other Brassicas.

*Other Species of Dipterous Larvae found accompanying the Maggots of H. brassicae in Cruciferous Plants.*

It does not lie within the scope of the present paper to give a detailed description of the other Dipterous pests of Cruciferous plants. Such description would entail a considerable amount of further study on a subject concerning which little is known. Nevertheless the writer considers it worth while to put on record the following facts concerning four other species of Dipterous larvae which were found associated with *H. brassicae* in Cruciferous plants.

*Phytomyza rufipes* Meig. (J. E. Collin det.). The larva of this insect was found in the upper part of the root of Brassicas and in the leaf bases; the largest number however was found tunnelling in the midribs of cauliflower and cabbage leaves. It is a smooth shining maggot about 6 mm. long when full grown. Each segment is strongly differentiated in the mature larva by a concentric ring of small spines surmounting each segmental swelling. The anterior spiracles stand well out from the body, and the posterior spiracles are directed downwards and backwards, forming a distinctive character by which the larva is easily identified. There is a complete absence of tubercles. These larvae were found causing considerable damage to the leaves of cauliflowers in autumn, the blackened borings in the midrib being distinctly visible from the exterior. More serious damage is occasionally done to young cauliflowers through these maggots boring in the head and causing blindness. Text-fig. 6 is a drawing of this larva. The puparium is to be found in large numbers in the soil in company with those of *H. brassicae*. It is seed-like in appearance, of a light brown colour and measures about 4 mm. in length. Most of the larval characteristics are still visible, particularly the anterior and posterior spiracles, and it is also possible to make out the segmental rings of larval spines. The adult is a small fly measuring 7 mm. across the wings, antennae very short and broad, yellow in the male, arista long and bare, head brownish with some white markings round eyes, bristles sparse. There is a small black raised area containing

spines situated between the eyes, legs and wing veins brown, thorax greyish with a number of long bristles sparsely set. Four pairs of long spines form two ill-defined rows down the centre of the dorsum, scutellum large, halteres yellowish white, abdomen greyish, thickly set with bristles. Female with black antennae, except for the extreme edge, which is yellow.

The eggs are inserted in the tissue of the leaf blade, the place of oviposition being marked by a small whitish ring. The larvae live mostly in the midrib and leaf bases, occasionally being found in the stem and growing points of young plants. Pupation takes place mostly in the soil, though puparia have occasionally been found in the leaf veins. There appear to be three or four generations in the season, adult flies being plentiful from May to October. The larva is heavily parasitised by *Dacnusa stramineipes* Halid. (Plate XXIII, fig. 4) which also parasitises the larva of *H. brassicae*. Theobald<sup>(15)</sup> refers to a fly which he bred from Crucifers as *Phytomyza flavicornis* Meig., this is probably the same insect. The second type of larva sometimes found associated with *H. brassicae* occurred on two occasions in the root of Brassicas. This larva (Fig. 7) resembles the cabbage-root maggot somewhat, but there is a considerable difference in the character of the larval skin. In *H. brassicae* it is smooth while that of the species under observation is muricate or shagreened, being entirely covered with minute spines. The caudal tubercles also differ in their arrangement, there being only one bifid tubercle. The writer failed to breed the flies from these larvae and so cannot state the species. Keilin, however<sup>(8)</sup>, records finding larvae of a different type feeding in the roots of Crucifers; these gave rise to a new species of fly which was named *Chortophila pilipyga* Villeneuve, and later re-named by Seguy<sup>(11)</sup> as *Hylemyia pilipyga*. It is possible that these rough-skinned larvae may be the maggots of the same insect.

The other two types of larvae are illustrated in Text-figs. 8, 9.

These two species of larvae were only present in cabbage stems in which there was a certain amount of decay and the writer considers them to have been living mainly upon decaying tissue and not to be of true phytophagous habit. The species illustrated in Text-fig. 8 was a slender and tapering larva with a rough muricate skin; it possessed a patch of spines (*sp.*) situate dorsally near the posterior end. The other species, Text-fig. 9, was less slender and tapering and possessed a smoother skin. The arrangement of the caudal tubercles in each species was characteristic and is illustrated in the text-figures. A line drawing of the larva of *H. brassicae* (Text-fig. 10) is also given for comparison with the other types.



*Parasites of H. brassicae.*

In the course of this study the following parasites were bred from *H. brassicae*:

## COLEOPTERA. STAPHYLINIDAE.

*Aleochara bilineata* Gyll. The larva of this beetle is predaceous upon the pupae of the cabbage root fly. According to Wadsworth<sup>(17)</sup> the first-stage beetle larva bores its way into the puparium of the Dipterous host and completes its metamorphosis inside, feeding upon the pupa, emerging finally as the adult beetle. The writer bred large numbers of this beetle from the pupae of *H. brassicae*, and there is no doubt that it constitutes a considerable check upon the development of the fly. Fowler<sup>(4)</sup> gives a description of this beetle and the closely allied species *Aleochara bipustulatus* (*nitida* Grav.). From the puparia containing this beetle larva, the writer bred a Hymenopterous insect which was parasitising *A. bilineata*. This insect has been determined by Waterston as the Proctotrupid, *Exallonyx ligatus* Nees. Plate XXIII, fig. 1, shows this Proctotrupid, the beetle larva from which the parasite was bred, and the ruptured pupal case of *H. brassicae*, in which both these insects were contained. The empty pupal case of *Exallonyx ligatus* still attached to the beetle larva may also be seen.

*Aleochara bipustulatus* (*nitida* Grav.). The writer found this smaller Staphylinid often associated with *H. brassicae* puparia at the roots of Brassicas. Although none were bred from puparia under observation, it is likely that this insect also is predaceous upon the pupae of *H. brassicae*.

## HYMENOPTERA. CYNIPIDAE.

*Cothonaspis rapae* Westw. This Cynipid is by far the commonest of all the parasites affecting *H. brassicae* and was bred in very large numbers. Over 30 per cent. of the second-generation pupae were parasitised by this insect. There appear to be two broods in the season, early in June a female was observed ovipositing in first-generation larvae. The second-generation adults commenced hatching on July 26th and continued to hatch during August and September. The times of emergence of the Cynipid were very variable, the parasites hatching from both first and second *H. brassicae* pupae as late as August 31st. The date of the last emergence of *Cothonaspis rapae* was October 4th. It was observed that the pupal period of this Cynipid seemed to last a considerable time, and this may further indicate that there are two generations only. Parasitised larvae of *H. brassicae* pupating on June 24th

gave rise to the parasite on July 29th, 30th and August 3rd. Plate XXIII, figs. 2 and 3, are photographs of *Cothonaspis rapae* Westw.

The next Hymenopterous parasite is a species of Braconid, *Dacnusa stramineipes* Halid. This insect chiefly parasitises the Phytomyzid larva (*P. rufipes*) but the writer bred it also on two occasions from the pupae of *H. brassicae*. Plate XXIII, fig. 4, is a photograph of this parasite.

In addition to the Hymenopterous parasites of *H. brassicae* here described, Wadsworth<sup>(18)</sup> records two further insects—the Ichneumons *Phygadeuon fumator* Grav. and *Atractodes tenebricosus* Grav. (*vestalis* Hal.). The writer has not succeeded in breeding these latter from pupae of *H. brassicae*.

#### DIPTERA. ANTHOMYIDAE.

*Phaonia trimaculata* Bouché. The larvae of this large Anthomyid fly occur in the roots of Cruciferous plants in association with the larvae of *H. brassicae* upon which they feed. These carnivorous larvae may be distinguished from the root maggots by their larger size and by the absence of the well-marked caudal tubercles. The pupae of *Phaonia* were also found by the writer together with those of *H. brassicae* in the soil surrounding the plant, distinguishable from the latter by their larger size. Bouché<sup>(1)</sup> first described this fly as *Anthomyia trimaculata*, and Wadsworth<sup>(17)</sup> recorded it for the first time in this country in 1915. Keilin<sup>(8)</sup> also has a note upon the carnivorous character of the larvae. The fly is light grey in colour with four black bands on the dorsum, legs black, and abdomen with a black dorsal band.

The last insect associated with *H. brassicae* to be described is the Tachinid fly *Onesia agilis* Meig. (*respillo* Rdi.) and its precise connection with the cabbage root maggot has not been determined.

The pupae of this fly were found on several occasions in company with those of *H. brassicae*. There seem to be two possible explanations concerning the activities of this fly, either, like the foregoing *Phaonia*, the larvae are carnivorous and prey upon the cabbage root maggot or they are, like many Tachinid larvae, feeders in decaying matter and are thus only secondary to the damage caused by *H. brassicae*.

As however there seems a definite association of this Tachinid with the cabbage root fly, the writer has considered it worth while to put the fact on record.

Plate XXIII, fig. 5, figures an interesting case of parasitism of the adult fly by some micro-organism, the exact nature of which has not yet been

determined. In the field a number of Anthomyid flies (not all *H. brassicae*) were observed to have an abnormal appearance about the abdomen. On capturing and examining the flies, they were found to be in a most interesting and unusual condition. Dorsally the abdomen presented a slightly rounded and swollen aspect, while ventrally there was a very large circular opening or crater with rounded lips leading into a cavity which occupied the greater part of the interior of the abdomen. In one or two cases there existed two of these crater-like orifices side by side in the abdomen of the same fly. There were no external signs of the disease visible in the fly other than the slight swelling referred to, and the crater-like opening or openings. It seems a remarkable thing that the flies so attacked were able to live an apparently normal life in spite of the fact that the abdomen was entirely occupied by a cancerous growth with its external orifice. Sections of these diseased flies revealed a cyst-like structure with an internal cavity opening to the exterior by the ventral orifice already referred to. Microscopical examination showed the presence of a micro-organism which, though unlike the parasitic *Empusa* in many ways, yet possessed some characteristics of that organism. At the same time minute spore-like bodies are present which cannot be attributed to *Empusa* and appear more characteristic of some Protozoan group (14).

#### SUMMARY.

A detailed account is given of the life history and parasites of *Hylemyia* (*Chortophila*) *brassicae*, the cabbage root fly. The various stages are described and figured. The egg is deposited in the vicinity of the roots of Cruciferous plants, in the heads of cauliflowers, and in autumn on the leaves and cut stem of Brassicas. The period of the egg stage lasts from 3 to 5 days, the larva when full grown is smooth, white in colour, and possesses a caudal corona of well-defined tubercles, one pair of which is bifurcate and serves as a means of identification. The maggots feed in the roots of Cruciferous plants, or in the heads of cauliflowers and turnips, while late in the autumn they may occasionally be found tunnelling in the midrib of *Brassica* leaves. The larval period lasts from 19 to 25 days. The puparium is reddish brown in colour and measures about 7 mm. in length. The length of the pupal stage is very variable and may occupy from an average of 16 days to 69 days. Pupation takes place in the soil in proximity to the plant. The adult is a dark grey insect somewhat resembling the house fly but is more bristly.

There are three generations in the season; as a general rule hibernation is in the soil in the pupal stage.

Four other types of larvae found associated with those of *H. brassicae* in Cruciferous plants are described, the most important being *Phytomyza rufipes* Meig.

A detailed description of the parasites of *H. brassicae* is given; these are the larva of the Staphylinid beetle *Aleochara bilineata* Gyll., the Cynipid *Cothonaspis rapae* Westw., the Braconid *Dacnusa stramineipes* Halid. and the Anthomyid fly *Phaonia trimaculata* Bouché. the larvae of which are carnivorous and feed upon *H. brassicae* larvae. There are also described a Proctotrupid, *Exallonyx ligatus* Nees., which is parasitic upon the larva of *Aleochara bilineata* Gyll. and a Tachinid fly, *Onesia agilis* Meig., which is associated with *H. brassicae*, although the exact relationship has not been determined.

An unusual form of parasitism of the adult fly by some kind of micro-organism is also figured and briefly described.

#### LITERATURE CITED.

- (1) BOUCHÉ, P. (1834). *Naturgeschichte der Insekten*.
- (2) CHITTENDEN, F. H. (1902). *U.S. Dept. of Agric. Div. of Entom. Bull.* 33, N.S., p. 80.
- (3) CURTIS, J. (1860). *Farm Insects*, p. 141.
- (4) FOWLER, CANON (1888). *British Coleoptera*. Vol. II. Staphylinidae, pp. 18-19.
- (5) GIBSON, A. and TREHERNE, R. C. (1916). The Cabbage Root Maggot and its Control in Canada. *Dept. of Agric. Entom. Branch. Bull.* No. 12. Ottawa.
- (6) GORDON HEWITT, C. (1907-8). *Journal Econ. Biology*, II, 56.
- (7) BEZZI, M. and STEIN, P. *Katalog. der Palaarktischen Dipteren*, III, 1907, 712.
- (8) KEILIN, D. (1917). *Parasitology*, IX, 440.
- (9) MILES, H. W. (1923). *Ann. Rept. Kirton Agric. Inst.* p. 48.
- (10) SCHOENE, W. J. (March, 1916). The Cabbage Root Maggot. Its Biology and Control. *New York Agric. Exp. Station Bull.* No. 419.
- (11) SEGUY, E. (1923). *Faune de France. Diptères Anthomyides*, p. 91. Paris.
- (12) SLINGERLAND, M. V. (1894). The Cabbage Root Maggot. *Cornell Univ. Agric. Exp. Station Bull.* 78, pp. 572-577.
- (13) SMITH, KENNETH M. (Nov. 1922). A Study of the Life-History of the Onion Fly (*Hylemyia antiqua* Meig.). *Ann. App. Biol.* IX, Nos. 3 and 4.
- (14) — (1927). An Unusual Form of Parasitism of an Anthomyid Fly. *Parasitology*, XIX, No. 2.
- (15) THEOBALD, F. V. (1905-6). *Rept. Econ. Zool. S.E. Agric. Coll.*
- (16) — (1910). *Ibid.* p. 100.
- (17) WADSWORTH, J. T. (June 1915). On the Life-History of *Aleochara bilineata* Gyll. A Staphylinid Parasite of *Chortophila brassicae* Bouché. *Journ. Econ. Biol.* X, Nos. 1 and 2.
- (18) — (1915). *Entom. Monthly Magazine*, p. 142.
- (19) — (July 1915). Notes on Some Hymenopterous Parasites. *Ann. App. Biol.* II, Nos. 2 and 3, 158-161.

EXPLANATION OF PLATE XXIII.

- Fig. 1. *Exallonyx ligatus* Nees. A Proctotrotypid, parasitic upon the larva of *A. bilineata*. In the figure the Proctotrotypid may be seen on the left, the parasitised beetle larva in the centre, and the pupal case of *H. brassicae* from which both insects have come is seen on the right. Note the empty pupal case of *E. ligatus* attached to the tail of the dead *Aleochara* larva.
- Fig. 2. The Cynipid parasite *Cothonaspis rapae* Westw. Male.  $\times 5$ .
- Fig. 3. *Cothonaspis rapae* Westw. Female.  $\times 5$ .
- Fig. 4. The Braconid parasite *Dacnusa stramineipes* Halid.  $\times 5$ .
- Fig. 5. Adult female of *H. brassicae* showing attack by a micro-organism. The large crater-like opening of the internal cyst is plainly shown.  $\times 5$ .

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Fig. 1.



Fig. 2.



Fig. 3.

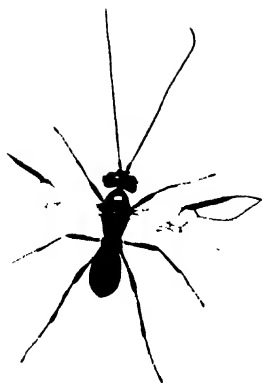


Fig. 4.



Fig. 5.



# STUDIES ON CONTACT INSECTICIDES

## PART VI. THE INSECTICIDAL ACTION OF THE FATTY ACIDS, THEIR METHYL ESTERS AND SODIUM AND AMMONIUM SALTS

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(With 9 Text-figures.)

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### INTRODUCTION.

It has been a common practice for many years to incorporate solutions of the salts of the higher fatty acids with insecticide and fungicide spray fluids, mainly with the object of increasing their wetting and spreading powers. The potash soaps of the liquid fatty acids such as oleic are invariably used, as their solutions are fluid at ordinary temperatures. In recent years however in the case of certain basic insecticides such as nicotine, the fatty acid has been combined directly with the organic base. It is generally known that, apart from their effect



upon the physical properties of the spray, these soap solutions themselves are toxic to insects.

The insecticidal properties of some of the fatty acids and soaps have recently been investigated by Siegler and Popenoe(1, 2). Their experiments were made on several species of aphids with the free acids, and with acid, neutral and alkaline soaps. The free acids were emulsified with a solution of glue, or, when used with water alone, by means of a colloid mill, and sprayed on to the insects with a small atomiser. They found that whereas the lower acids showed no great toxicity to aphids, caproic acid showed "marked toxicity," and caprylic acid killed 90 per cent. at a dilution of 1 in 500, with only slightly decreased toxicity at 1 in 1000. Capric acid killed 99 per cent. and lauric acid 92 per cent. at 1 in 1200. Myristic acid was less toxic. The figures do not all apply to the same species of aphid. Rapid and complete paralysis of the insects was noted and the hypothesis is put forward that "the fatty acids in a crystalloid state penetrate the body walls and tracheae, thus gaining immediate haemolytic action on the haemolymph and body cells"(2). Further, these investigators consider that the toxicity of soaps is chiefly due to the action of free fatty acid liberated by hydrolysis.

Siegler and Popenoc have, so far as we are aware, published only brief accounts of their experiments, without any detailed figures. The fatty acid series appears particularly suitable for the study of change of toxicity with increase of molecular complexity, for each member differs from the next by one  $\text{CH}_2$  group only, and all except formic acid contain a chemically reactive carboxyl group united to a comparatively unreactive hydrocarbon chain. This series of acids up to stearic was therefore included in our survey of the insecticidal action of various groups of organic compounds.

The data presented here indicate that within a wide range there is a steady increase in toxic action, as the series is ascended. There is moreover a gradation in certain physical properties, and the question arises whether the change in toxic action is not determined largely by one or more of these dependent properties rather than directly by the molecular complexity of a relatively inert hydrocarbon chain. A preliminary attempt is therefore made to trace out relationships between certain physical factors and the toxic action among these acids. The carboxyl group is readily esterified and saponified, giving compounds with new sets of properties, and the methyl and benzyl esters and the sodium and ammonium salts of a number of these acids have been investigated. In each case interesting quantitative changes in toxic action

were noted, indicating that both the carboxyl group and the hydrocarbon chain play a significant part in the toxic effect.

The introduction of a double bond in the hydrocarbon chain brings about certain important changes in the chemical and physical properties, and the determination of its effect seemed of some importance. Two unsaturated acids (undecenoic and oleic) were therefore included in the tests.

The methods employed for the determination of insecticidal values have been dealt with in previous papers (3, 4, 5), and a brief résumé is given in Part V of this series of papers (this vol., p. 217).

#### EXPERIMENTS WITH *APHIS RUMICIS*.

In the case of the salts dealt with in this paper, which themselves cause a marked lowering of surface tension, we have made a departure from the usual technique and have used a 0.5 per cent. solution of saponin (in place of 1 per cent.) for dilution. The sodium salts of lauric, palmitic and stearic acids proved difficult to spray on account of the tendency of their solutions to form gels; this was got over in the case of sodium laurate by spraying at a slightly higher temperature, but no data were obtained with sodium palmitate and stearate.

The samples of the fatty acids available were not in all cases chemically pure. The acid content of each was therefore determined by titration and the necessary corrections made where the acid content was low enough to introduce a significant error. Except in the cases of lauric and undecenoic acids, the corrections required were quite small. The figures are given in Table I.

The salts were prepared by dissolving known amounts of each of the acids in the smallest quantity of alcohol necessary to bring to a clear solution and adding caustic soda or ammonia, until in the case of the sodium soaps the solution was slightly alkaline, and in that of the ammonium salts distinctly alkaline, to phenol phthalein. The solutions were then warmed, allowed to cool, and a little more alkali added if the colour did not return on cooling. No attempt was made to bring each solution to the same pH value.

After spraying, the insects are always placed within reach of fresh bean foliage and are examined on the two (or if necessary, three) following days. At each examination counts are made under four headings: "unaffected," "slightly affected," "moribund," and "dead." With the compounds discussed in this paper, the figures for the first day's count

are tabulated, except in the few cases when recoveries occurred after a further 24 hours. In the tables, except in the cases of unimportant compounds, the data for the four categories are given, but in the diagrams the sums of the figures in the last two categories only are plotted against the concentration.

It is true that the diagrams do not always show the finer changes in toxicity with change in concentration so well as the figures in the tables. They do however indicate satisfactorily the difference in toxicity existing between the compounds and, as the curves are not in any case suitable for purposes of statistical analysis, it has not been thought worth while to introduce a more detailed and laborious method of graphing the results.

In a previous paper<sup>(3)</sup> we have stated that the concentrations yielding a mortality of 50 per cent. are the most suitable for purposes of comparison as between the different compounds. It was noted, however, that with many of the fatty acids a slight decrease in concentration resulted in a profound quantitative change in toxic action and some of the curves decline so steeply that exploration about the region of the 50 per cent. point was impracticable; therefore, when comparisons were made between partition coefficients or the number of carbon atoms in the molecule and toxicity, we chose the lowest concentrations yielding 100 per cent. moribund and dead for the purpose, and have attempted, among the more highly toxic compounds, to determine this value with as great an accuracy as possible for purposes of correlation.

#### *The Fatty Acids.*

In Table I the results obtained for all the concentrations of all the acids tested are set out. In Diagram 1, in order to avoid too many inter-crossings, the values for the saturated, but not for the unsaturated acids are graphed.

An inspection of Table I and Diagram 1 shows that formic acid, while only slightly toxic, is more so than acetic. Formic acid cannot be regarded as a typical fatty acid, as in addition to the constitution of an organic acid it has an aldehydic structure which confers reducing properties and probably increases toxicity. Both Table and Diagram show that toxic properties steadily increase from acetic to decylic and undecylic acids. The difference in toxic values between propionic and butyric acids is only a small one, but there occurs a large gap between butyric and valerianic acids. Diagram 1 shows no significant difference in toxicity between decylic and undecylic acids, but dodecylic (lauric) and

Table I.

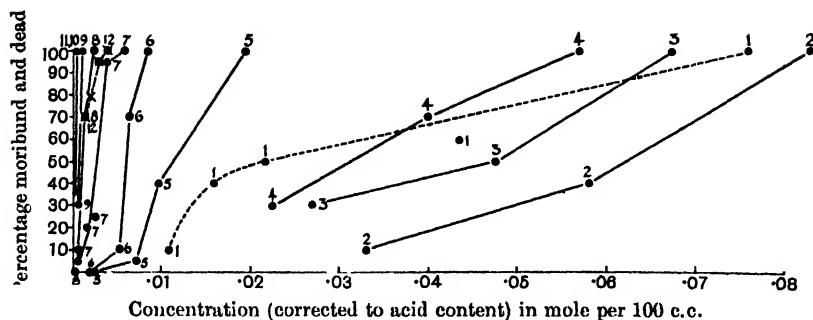
*Toxicities of the Fatty Acids to A. rumicis (1st Series).*

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name of acid	No. of tests	Concentration of acid		N %	S %	M %	D %	M and D %
		Gm. per 100 c.c.	Mole per 100 c.c.					
Formic. (HCOOH.	1	5.0	0.109	—	—	—	100	100
No. of carbon atoms 1.	1	3.5	0.076	—	—	—	100	100
Mol. wt. 46.02.	1	2.0	0.0435	20	20	—	60	60
% acid content 97.8)	2	1.0	0.022	40	10	25	25	50
	1	0.75	0.016	30	30	—	40	40
	1	0.5	0.0108	90	—	—	10	10
Acetic. (CH <sub>3</sub> COOH.	1	5.0	0.083	—	—	50	50	100
No. of carbon atoms 2.	1	3.5	0.058	30	30	20	20	40
Mol. wt. 60.04.	1	2.0	0.033	50	40	10	—	10
% acid content 98.5)	2	1.0	0.0166	75	15	5	5	10
	1	0.5	0.0083	90	10	—	—	—
Propionic. (C <sub>3</sub> H <sub>7</sub> COOH.	1	5.0	0.067	—	—	10	90	100
No. of carbon atoms 3.	1	3.5	0.047	30	20	30	20	50
Mol. wt. 74.06.	1	2.0	0.027	60	10	30	—	30
% acid content 96.75)	2	1.0	0.0135	90	5	5	—	5
	1	0.5	0.0067	100	—	—	—	—
Butyric. (C <sub>4</sub> H <sub>9</sub> COOH.	1	5.0	0.057	—	—	50	50	100
No. of carbon atoms 4.	1	3.5	0.04	—	30	70	—	70
Mol. wt. 88.08.	1	2.0	0.022	20	50	20	10	30
% acid content 96.6)	1	1.0	0.0113	50	20	20	10	30
	1	0.75	0.0085	80	—	10	10	20
	1	0.25	0.0028	100	—	—	—	—
Valerianic (normal). (C <sub>5</sub> H <sub>11</sub> COOH.	1	5.0	0.049	—	—	—	100	100
No. of carbon atoms 5.	1	3.5	0.034	—	—	—	100	100
Mol. wt. 102.1.	1	2.0	0.0196	—	—	40	60	100
% acid content not determined)	1	1.0	0.01	20	40	40	—	40
	2	0.75	0.0073	80	15	—	5	5
	1	0.25	0.0025	100	—	—	—	—
Caproic (hexylic) (isobutyl acetic). (C <sub>6</sub> H <sub>13</sub> COOH.	1	2.0	0.017	—	—	—	100	100
No. of carbon atoms 6.	1	1.0	0.0086	—	—	30	70	100
Mol. wt. 116.1.	2	0.75	0.0064	30	—	60	10	70
% acid content 98.75)	1	0.5	0.0043	70	20	10	—	10
	2	0.25	0.0021	85	15	—	—	—
Oenanthalic (heptylic). (C <sub>7</sub> H <sub>15</sub> COOH.	3	0.75	0.0057	—	—	—	100	100
No. of carbon atoms 7.	2	0.5	0.0038	5	—	45	50	95
Mol. wt. 130.1.	2	0.35	0.0027	40	35	15	10	25
% acid content 97.2)	2	0.2	0.0015	45	35	20	—	20
	2	0.1	0.00077	80	10	10	—	10
Caprylic (octylic). (C <sub>8</sub> H <sub>17</sub> COOH.	2	0.75	0.0052	—	—	—	100	100
No. of carbon atoms 8.	2	0.5	0.0035	—	—	—	100	100
Mol. wt. 144.2.	2	0.35	0.0024	—	—	5	95	100
% acid content 99.15)	2	0.22	0.0014	—	30	55	15	70
	2	0.1	0.0007	95	—	—	5	5
	2	0.05	0.00035	95	5	—	—	—

Table I (continued)

Name of acid	No. of tests	Concentration of acid		N %	S %	M %	D %	M and D %
		Gm. per 100 c.c.	Mole per 100 c.c.					
Pelargonic (nonylic).	1	0.68	0.0043	—	—	—	100	100
(C <sub>9</sub> H <sub>17</sub> COOH.	2	0.45	0.0028	—	—	15	85	100
No. of carbon atoms 9.	2	0.31	0.002	—	—	10	90	100
Mol. wt. 158.1.	2	0.18	0.0011	—	—	35	65	100
% acid content 91.55)	2	0.09	0.00057	35	35	20	10	30
	2	0.045	0.00028	80	20	—	—	—
Capric (decylic).	1	0.7	0.004	—	—	—	100	100
(C <sub>10</sub> H <sub>19</sub> COOH.	2	0.46	0.0027	—	—	10	90	100
No. of carbon atoms 10.	2	0.32	0.0019	—	—	20	80	100
Mol. wt. 172.2.	2	0.186	0.00117	—	—	40	60	100
% acid content 92.85)	2	0.093	0.00054	—	—	65	35	100
	2	0.046	0.00027	25	40	35	—	35
	2	0.023	0.00013	65	30	5	—	5
Undecylic.	1	0.7	0.0037	—	—	—	100	100
(C <sub>11</sub> H <sub>21</sub> COOH.	2	0.46	0.0024	—	—	—	100	100
No. of carbon atoms 11.	2	0.32	0.0017	—	—	—	100	100
Mol. wt. 186.3.	2	0.186	0.00098	—	—	40	60	100
% acid content 92.5)	2	0.093	0.00049	—	—	85	15	100
	2	0.046	0.00024	30	35	20	15	35
	2	0.023	0.00012	60	40	—	—	—
Lauric (dodecylic).	1	0.8	0.004	—	—	30	70	100
(C <sub>12</sub> H <sub>23</sub> COOH.	4	0.6	0.003	2.5	2.5	72.5	22.5	95
No. of carbon atoms 12.	3	0.4	0.002	10.3	10.3	44.8	34.5	79
Mol. wt. 200.2.	3	0.28	0.0014	10	20	43.3	26.7	70
% acid content 80)	3	0.16	0.0008	13.3	36.7	30	20	50
	3	0.08	0.0004	66.7	20	6.6	6.6	13
Tridecylic.	5	4.5-0.67	0.021 0.0031	—	—	90	10	100
(C <sub>13</sub> H <sub>25</sub> COOH.	1	0.45	0.0021	—	—	90	10	100
No. of carbon atoms 13.	1	0.36	0.0017	—	10	40	50	90
Mol. wt. 214.2.	1	0.315	0.0015	—	—	100	—	100
% acid content 90)	1	0.27	0.00126	—	10	70	20	90
	1	0.18	0.00084	40	30	30	—	30
	1	0.09	0.00042	80	—	10	10	20
Myristic (tetradecylic).	Not materially toxic at a concentration of 5.0 % = 0.02 mole per 100 c.c.							
(C <sub>14</sub> H <sub>27</sub> COOH.								
No. of carbon atoms 14.								
Mol. wt. 228.								
% acid content 92.1)								
Palmitic C <sub>15</sub> H <sub>31</sub> COOH and Stearic C <sub>17</sub> H <sub>35</sub> COOH	Not materially toxic at a concentration of 5.0 % (0.0195 and 0.0175 mole per 100 c.c. respectively.							
Undecenoic.	5	0.645-0.3	0.0035-0.0016	—	—	—	—	100
(C <sub>11</sub> H <sub>19</sub> COOH.	2	0.17	0.00092	—	—	90	10	100
No. of carbon atoms 11.	2	0.086	0.00046	15	—	60	25	85
Mol. wt. 184.27.	2	0.043	0.00023	5	55	30	10	40
% acid content 85.7)	1	0.021	0.00011	90	—	—	10	10
Oleic.	1	1.0	0.0035	—	—	—	100	100
(C <sub>17</sub> H <sub>33</sub> COOH.	2	0.75	0.0026	—	5	10	85	95
No. of carbon atoms 18.	1	0.5	0.0018	30	—	30	40	70
Mol. wt. 282.36.	1	0.35	0.0012	50	30	10	10	20
% acid content 97.7)	1	0.2	0.0007	50	30	20	—	20



The acids are specified by the number of carbon atoms in the molecule (see Table I).

Diagram 1. Toxicity of the fatty acids to *A. rumicis*.

tridecylic acids decline in toxicity to a small but probably significant extent. The acids higher than tridecylic are distinctly less toxic, myristic, palmitic and stearic showing no toxic action at any of the concentrations tested.

In order to fix more accurately the lowest concentration giving 100 per cent. mortality and to determine more exactly the change in toxicity with decreasing concentration, a fresh series of experiments with the more highly toxic acids was made, the concentrations used being brought as near together as conveniently possible. To avoid the possibility of effects due to secondary factors, benzene was not used in this series, the solid acids being dispersed in saponin solution in extremely fine condition. Lauric acid could not be used in this way as the finer particles aggregated at too great a rate. The results are given in Table II and Diagram 2 which is plotted on a larger scale than Diagram 1. In Diagram 2 the data for dodecylic (lauric) and tridecylic acids are taken from Table I.

The results of these experiments are in close agreement with those obtained previously and confirm the increase in toxicity with molecular weight from the 5 carbon-atom acid to the 11 carbon-atom acid, and demonstrate that on passing from decylic to undecylic acid there is a slight increase in toxicity.

The results agree well with the statement of Siegler and Popenoe(1) that "the toxicity of the fatty acids increases with the molecular weight, at least to a certain point not yet definitely determined. A practical amount of toxicity was reached with the sixth carbon-atom, the peak of toxicity apparently lying at or slightly above the  $C_{10}$  point."

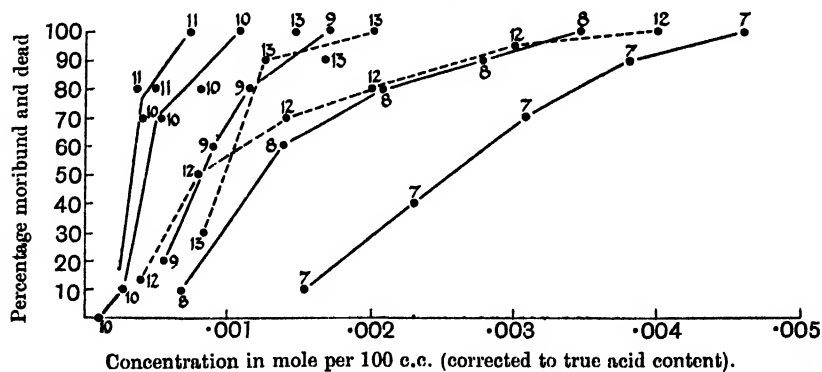
Table I shows the results obtained with the two unsaturated acids, undecenoic and oleic. The former is as toxic as the saturated acids,

Table II.

*Toxicities of the Fatty Acids to A. rumicis (2nd Series).*

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name of acid	Concentration of acid		N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.					
Oenanthic (heptylic). Acid content 97.2 %	0.6	0.0046	—	—	—	100	100
	0.5	0.0038	—	10	20	70	90
	0.4	0.0031	10	20	30	40	70
	0.3	0.0023	50	10	40	—	40
	0.2	0.0015	90	—	—	10	10
Caprylic (octylic). Acid content 99.1 %	0.5	0.0034	—	—	—	100	100
	0.4	0.0028	—	10	10	80	90
	0.3	0.0021	—	20	40	40	80
	0.2	0.0014	20	20	60	—	60
	0.1	0.0009	80	10	—	10	10
Pelargonic (nonylic). Acid content 91.5 %	0.36	0.0023	—	—	10	90	100
	0.27	0.0017	—	—	30	70	100
	0.18	0.0011	—	20	70	10	80
	0.135	0.00085	—	40	50	10	60
	0.09	0.00057	20	60	20	—	20
	0.067	0.00042	50	30	10	—	10
Capric (decylic). Acid content 92.85 %	0.186	0.001	—	—	30	70	100
	0.14	0.0008	—	20	50	30	80
	0.093	0.00054	—	30	70	—	70
	0.07	0.0004	10	20	50	20	70
	0.046	0.00027	70	20	—	10	10
	0.023	0.00013	90	10	—	—	—
Undecylic. Acid content 92.5 %	0.186	0.00098	—	—	10	90	100
	0.14	0.00074	—	—	30	70	100
	0.093	0.00049	—	20	60	20	80
	0.07	0.00037	—	20	80	—	80
	0.046	0.00024	70	30	—	—	—
	0.023	0.00012	80	—	20	—	20

The acids are specified by the number of carbon atoms in their molecules (*e.g.* 7 = heptylic).Diagram 2. Toxicities of the fatty acids (heptylic to dodecylic) to *A. rumicis*.

undecylic and decylic, the slight differences being scarcely significant. Oleic acid is less toxic than undecenoic acid.

*The Sodium and Ammonium Salts of the Fatty Acids.*

Solutions of the sodium and ammonium salts of the fatty acids were prepared in the manner already described (p. 333) and dilutions made up in the usual way, except that the strength of the saponin solution was reduced to 0.5 per cent. It seemed advisable for compounds like the soaps, in which surface activity is so pronounced, to reduce the strength of the wetting reagent. In two sets of experiments carried out with sodium oleate, with and without the addition of saponin, the results obtained were in very close agreement; in each case a concentration of 0.75 per cent. (reckoned as acid) resulted in a mortality of 100 per cent. and 0.5 per cent. gave 90 per cent. of deaths.

The data for the *sodium salts* are not given completely except in the case of sodium oleate. Conversion of the acid to the sodium salt greatly reduces toxicity as a rule; thus, stating the results in terms of the acid, pelargonic acid (nonyllic) was completely toxic at a concentration of 0.2 per cent. whereas the corresponding sodium soap was scarcely toxic at 1 per cent.; capric acid (decylic) and undecylic acid were toxic at 0.1 per cent., the sodium salts barely toxic at 1.0 per cent. With lauric acid (dodecylic) and myristic acid (tetradecylic), the effect of converting to the sodium salts was not so pronounced, sodium laurate being scarcely less toxic, and sodium myristate apparently more toxic, than the corresponding acid; but in both these cases, particularly with the myristate, there was a tendency for the insects to be glued down by the material, with consequent injury. Further, if the figures for sodium oleate are compared with those for sodium undecenoate, it will be seen that the effect of saponification in reducing toxicity is less marked among the highest members of the series; thus, whereas undecenoic acid is completely toxic at 0.2 per cent., the sodium soap is not toxic at all at a concentration (calculated as acid) of 0.5 per cent. and scarcely toxic at 1.0 per cent. Oleic acid and sodium oleate on the other hand show hardly a significant difference and, if anything, the soap is slightly more toxic. It was unfortunately impossible to compare the sodium palmitates and stearates with the corresponding acids, as their solutions are gels at ordinary temperatures even at fairly low dilutions and were not suitable for spraying.

The results obtained with the *ammonium salts* of the acids, ranging from caproic to stearic, are expressed in Table III, which also includes



sodium oleate; and in Diagram 3 the results for the ammonium salts from caproic to lauric acid are plotted.

Table III.

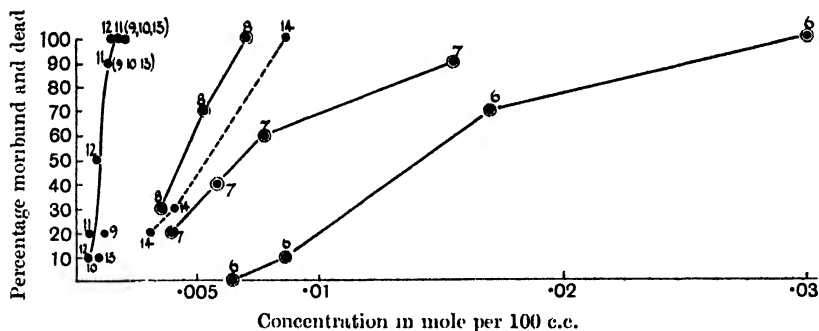
*Toxicities of the Ammonium Salts of the Fatty Acids to A. rumicis.*

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name	Concentration of acid		N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.					
Ammonium caproate (hexylate)	3.5	0.03	—	—	30	70	100
	2.0	0.017	30	—	10	60	70
	1.0	0.0086	90	—	—	10	10
	0.75	0.0064	90	10	—	—	—
Ammonium ocanthate (heptylate)	2.0	0.0154	10	—	—	90	90
	1.0	0.0077	20	20	20	40	60
	0.75	0.0057	40	20	30	10	40
	0.5	0.0038	80	—	10	10	20
	0.35	0.0027	70	10	—	20	20
	0.2	0.0015	100	—	—	—	—
Ammonium caprylate (octylate)	1.0	0.007	—	—	—	100	100
	0.75	0.0052	20	10	20	50	70
	0.5	0.0035	60	10	10	20	30
	0.35	0.0024	50	20	—	30	30
	0.2	0.0014	100	—	—	—	—
Ammonium pelargonate (nonylate)	0.9	0.0054	—	—	—	100	100
	0.68	0.0043	—	—	—	100	100
	0.45	0.0028	—	—	10	90	100
	0.31	0.002	—	—	40	60	100
	0.225	0.0014	—	10	20	70	90
	0.18	0.0011	50	30	10	10	20
	0.09	0.00057	80	20	—	—	—
Ammonium caprate (decylate)	0.46	0.0027	—	—	10	90	100
	0.35	0.002	—	—	30	70	100
	0.23	0.0013	—	10	50	40	90
	0.093	0.00054	80	10	10	—	10
Ammonium undecylate	0.93-0.46	0.0049-0.0024					100
	0.32	0.0017	—	—	10	90	100
	0.23	0.0012	—	10	30	60	90
	0.093	0.00049	30	50	10	10	20
Ammonium laurate (dodecylate)	2.0-0.4	0.01-0.002					100
	0.28	0.0014	—	—	40	60	100
	0.16	0.0008	40	10	50	—	50
	0.08	0.0004	60	30	—	10	10
	0.04	0.0002	80	20	—	—	—
Ammonium tridecylate	0.45	0.0021	—	—	40	60	100
	0.31	0.0014	—	20	30	50	80
	0.18	0.00084	80	10	—	10	10
	0.09	0.00042	90	10	—	—	—
Ammonium myristate (tetradecylate)	1.84	0.008	—	—	30	70	100
	0.92	0.004	50	20	10	20	30
	0.69	0.003	60	20	—	20	20
	0.46	0.002	60	20	10	10	20
	0.32	0.0014	90	10	—	—	—

Table III (continued).

Name	Concentration of acid		N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.					
Ammonium palmitate	Not toxic, at a concentration of 2.0 % of the acid = 0.0078 mole per 100 c.c.						
Ammonium stearate	Not toxic at a concentration of 2.0 % of the acid = 0.007 mole per 100 c.c.						
Ammonium undecenoate	0.63	0.005	—	—	—	100	100
	0.43	0.0023	—	10	10	80	90
	0.21	0.0011	—	10	20	70	90
	0.086	0.00046	60	30	—	10	10
Ammonium oleate	2.5-0.75	0.0088-0.0026	—	—	—	—	100
	0.5	0.0018	—	—	—	100	100
	0.35	0.0012	20	—	—	80	80
	0.2	0.0007	20	10	—	70	70
	0.1	0.00035	100	—	—	—	—
Sodium oleate	2.5 and 1.0	0.0088-0.0035	—	—	—	—	100
	0.75	0.0026	—	—	10	90	100
	0.5	0.0018	5	5	5	85	90
	0.35	0.0012	20	10	—	70	70
	0.2	0.0007	40	10	—	50	50
	0.1	0.00035	40	30	—	30	30
Ammonia control	Not toxic, at 9.0 %						
0.5 and 1.0 % saponin solutions	Not toxic						
Alcohol and 0.5 % saponin solutions	Not toxic						



The compounds are specified by the number of carbon atoms in the acid (e.g. the line drawn through 6 indicates the results for ammonium caproate (hexylate)).

Diagram 3. Toxicities of the ammonium salts of the fatty acids to *A. rumicis*.

The neutralisation of the acid radical with ammonia decreases toxicity in certain of the acids. The reduction of toxicity due to neutralisation with ammonia is again less in the case of the higher fatty acids from nonylic upwards, ammonium tridecylate being of the same order of

toxicity as tridecylic acid and ammonium myristate markedly more toxic than myristic acid. It is probable that the toxicity of the ammonium salts is at least partly determined by the liberation by hydrolysis of the free fatty acids in a very finely divided state.

The ammonium salt of myristic acid (tetradecylic) is, however, less toxic than the ammonium salts of the acids of lower molecular weight (nonylic to tridecylic), while ammonium palmitate and stearate, at the highest concentration at which they could be conveniently tested, show no toxicity. As the same relationships are found amongst the acids themselves, it would appear that in this series molecular complexity, or some property depending on it, limits toxic action.

#### *The Methyl Esters of the Fatty Acids.*

The results obtained for the methyl esters of the fatty acids are given in Table IV and Diagram 4.

With the exception of methyl caprate, the toxicities are uniformly distinctly lower than those of the acids or the ammonium salts. Our tests may have exaggerated the toxicity of methyl caprate. Our sample was a very small one and the data in this case are probably not so good as in the rest of the tests. Nevertheless our sample of methyl caprate proved somewhat less toxic than the acid, mole for mole. The whole of the results demonstrate that the effect of methylation is to lower toxicity, and that there is an increase in toxicity as one ascends the series from methyl caproate (hexylate) to methyl caprate (decylate); methyl undecylate was not available, but methyl undecenoate shows a slight increase in toxicity over the caprate but is slightly less toxic than the corresponding acid, mole for mole. Methyl laurate (dodecylate) is less toxic than its immediate predecessors in the series and methyl stearate is not toxic at any concentration up to 5.0 per cent. (= 0.017 mole per 100 c.c.). The other noteworthy feature of the series is the comparatively high toxicity of methyl formate; this compound proved completely toxic at 0.083 mole per 100 c.c., whereas the acetate, propionate, butyrate and valerianate are not toxic at very high concentrations.

Certain of the benzyl esters were tested. The results suggest that where the acid is of small molecular weight the benzyl group has a preponderating effect upon toxicity, whereas with the acids of higher molecular weight the effect of this radical diminishes. As certain important benzyl esters were not available, the data with regard to this series is hardly sufficient to warrant further discussion at this stage.

Table IV.

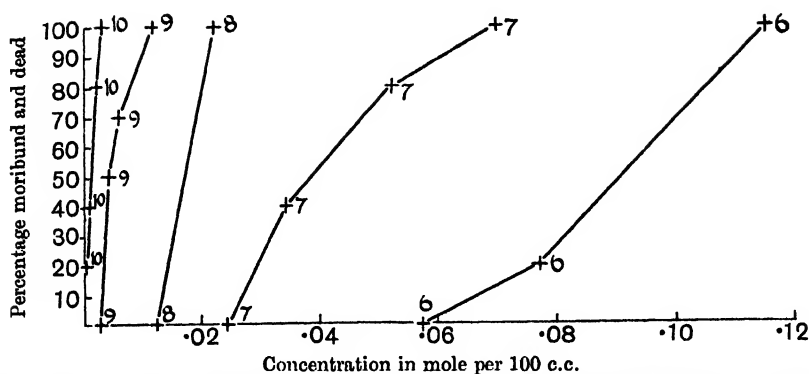
*Toxicities of the Methyl Esters of the Fatty Acids to A. rumicis.*

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name	Concentration of ester		N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per 100 c.c.*					
Methyl formate. ( $\text{H} \cdot \text{COOCH}_3$ )	5.0	0.083	—	—	—	100	100
Mol. wt. = 60)	3.5	0.058	40	20	10	30	40
	2.0	0.033	50	10	10	30	40
Methyl acetate (M.W. 74)	Not toxic, at a concentration of 20 %						
„ propionate ( „ 88)		„	„	„	„		
„ butyrate ( „ 102)		„	„	„	„		
„ valerianate ( „ 116)		„	„	15 %	„		
Methyl caproate (hexylate) ( $\text{C}_6\text{H}_{11}\text{COOCH}_3$ )	15	0.115	—	—	50	50	100
Mol. wt. = 130.1)	10	0.077	—	80	20	—	20
Methyl oenanthe (heptylate) ( $\text{C}_7\text{H}_{13}\text{COOCH}_3$ )	15	0.104	—	—	—	100	100
Mol. wt. = 144.1)	10	0.069	—	—	20	80	100
	7.5	0.052	—	20	20	60	80
	5.0	0.034	10	50	—	40	40
	3.5	0.024	30	70	—	—	—
Methyl caprylate (octylate) ( $\text{C}_8\text{H}_{15}\text{COOCH}_3$ )	10-5.0	0.063-0.031	—	—	—	—	100
Mol. wt. = 158.1)	3.5	0.022	—	—	50	50	100
	2.0	0.012	60	10	20	10	30
Methyl pelargonate (nonylate) ( $\text{C}_9\text{H}_{17}\text{COOCH}_3$ )	2.0	0.011	—	—	10	90	100
Mol. wt. = 172.2)	1.0	0.006	—	30	60	10	70
	0.75	0.0043	30	20	50	—	50
	0.5	0.003	50	50	—	—	—
Methyl caprate (decylate) ( $\text{C}_{10}\text{H}_{19}\text{COOCH}_3$ )	2.0 and 1.0	0.0107 and 0.0053	—	—	—	—	100
Mol. wt. = 186.2)	0.75	0.004	—	—	70	30	100
	0.5	0.0027	—	—	90	10	100
	0.35	0.0019	10	10	80	—	80
	0.2	0.001	30	30	40	—	40
	0.1	0.0005	70	10	20	—	20
	0.05	0.00027	80	20	—	—	—
Methyl laurate (dodecylate) ( $\text{C}_{12}\text{H}_{25}\text{COOCH}_3$ )	5.0 and 3.5	0.023 and 0.016	—	—	—	—	100
Mol. wt. = 214.25)	2.0	0.0093	—	—	—	100	100
	1.0	0.0046	10	20	10	60	70
	0.75	0.0035	20	10	—	70	70
	0.5	0.0023	—	—	—	—	30†
	0.25	0.0016	70	10	20	—	20
Methyl stearate ( $\text{C}_{17}\text{H}_{35}\text{COOCH}_3$ )	Not toxic at concentration of 5.0 % = 0.0176 mole per 100 c.c.						
Methyl undecenoate ( $\text{C}_{10}\text{H}_{19}\text{COOCH}_3$ )	2.0-0.75	0.01-0.004	—	—	—	—	100
Mol. wt. = 198.2)	0.5	0.0025	—	—	40	60	100
	0.35	0.0017	—	10	90	—	90
	0.2	0.001	—	20	80	—	80
	0.1	0.0005	40	60	—	—	—
Methyl oleate ( $\text{C}_{17}\text{H}_{33}\text{COOCH}_3$ )	5.0	0.017	—	—	—	100	100
Mol. wt. = 296.3)	3.5	0.012	—	—	30	70	100
	2.0	0.0067	20	—	50	30	80
	1.0	0.0033	40	20	30	10	40
	0.75	0.0025	50	40	—	10	10

\* The concentrations in moles are the same whether expressed as acid or ester.

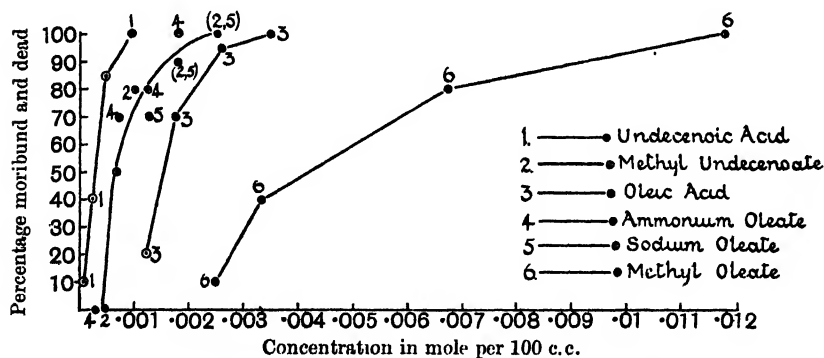
† Experiment spoilt owing to escape of insects.



The methyl esters are specified by the number of carbon atoms in the acid (e.g. 6 = methyl caproate (hexylate)).

Diagram 4. Toxicities of the methyl esters of the fatty acids to *A. rumicis*.

Diagram 5 sets out the results obtained with the unsaturated acids (undecenoic and oleic) and their ammonium salts and methyl esters. Ammonium undecenoate is omitted in order not to obscure the diagram; it is slightly less toxic than the acid. The toxicities of methyl unde-



Ammonium undecenoate is not included; it was somewhat less toxic than undecenoic acid.

Diagram 5. Toxicities of undecenoic and oleic acids, their ammonium and sodium soaps and methyl esters, to *A. rumicis*.

cenoate, ammonium and sodium olcate at different concentrations (points 2, 4, 5) can be substantially represented by one curve. The diagram shows methyl undecenoate (2) less toxic than its acid, and methyl olcate less toxic than oleic acid. This acid is slightly less toxic than either its sodium or ammonium salts.

Diagram 6 shows the relationship between the insecticidal values of the acids, their ammonium salts and methyl esters and the molecular

weight of the respective acid which is represented by the number of carbon atoms present. The comparisons are made at concentrations giving 100 per cent. moribund and dead<sup>1</sup>.

The diagram clearly indicates the increases in toxicity with molecular complexity, formic acid and its derivatives being exceptional throughout the three series; and in addition shows that the ammonium salts containing 6, 7 and 8 carbon atoms are less toxic than the corresponding

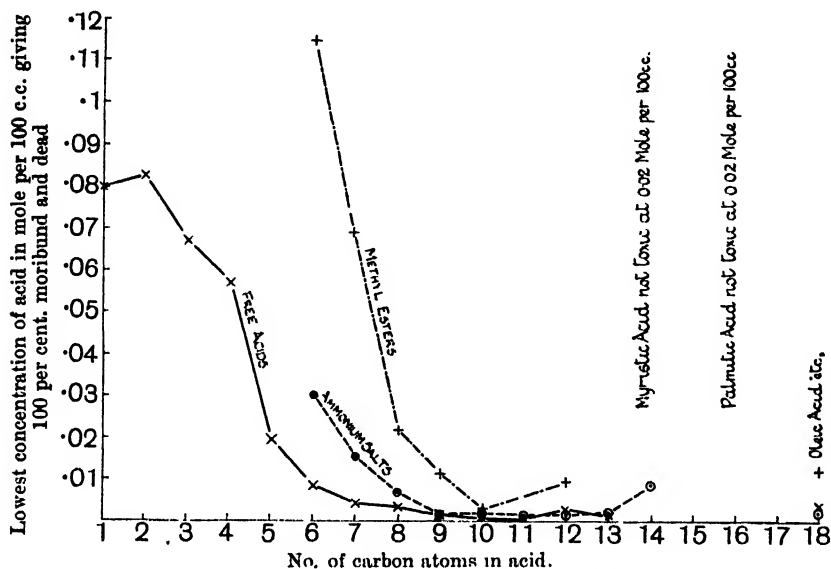


Diagram 6. Showing relationship between toxicity to *A. rumicis* of the fatty acids, their ammonium salts and methyl esters, and the number of carbon atoms in the molecule of each acid.

acids, but that from this point onwards to the 13 carbon-atom acid the toxicity of the ammonium salt approximates to that of its acid, and at 14 (myristic) and 18 (oleic) the ammonium salt is more toxic. The methyl esters are uniformly less toxic than the corresponding acids. Speaking generally, these facts indicate that both the hydrocarbon chain and the carboxyl group play a significant part in determining the intensity of the toxic action of this group.

<sup>1</sup> In the case of ammonium oenanthate (heptylate), the percentage giving a death rate of 90 per cent. has been taken, the value for exactly 100 per cent. not having been obtained.

EXPERIMENTS WITH EGGS OF *SELENIA TETRALUNARIA*.

A series of experiments was carried out to ascertain whether the fatty acids showed any toxicity to eggs of the moth *Selenia tetralunaria* Hüfn. The general method of procedure has been described in earlier papers. The acids were tested at concentrations ranging from 5.0 per cent. to 0.25 per cent., and the liquids for spraying were prepared by dissolving the acid in a small amount of benzene and emulsifying with the required amount of a 1 per cent. solution of saponin in water. Control sprayings were made with benzene (5–50 per cent.) and saponin solutions. The results are summarised in Table V.

Table V.

*Toxicities of the Fatty Acids to the Eggs of Selenia tetralunaria.*

Name of acid	Concentration in gm. per 100 c.c.	No. of tests	No. of eggs sprayed	No. of eggs not hatching	% eggs not hatching	% viable eggs killed (allowing for control)
Controls	—	14	1676	68	4.04	—
Caproic	2.0–0.25	4	480	18	3.75	0
Oenanthic	2.0	1	169	96	56.8	54.8
„	1.0–0.25	3	289	14	4.5	0
Caprylic	2.0	1	163	108	66.2	64.65
„	1.0	1	102	15	14.7	11.1
„	0.5	1	100	10	10.0	6.2
„	0.25	1	99	3	3.03	0
Pelargonic	2.0	1	127	102	80.3	79.3
„	1.0–0.25	3	325	14	4.3	0
Capric	2.0–0.25	4	446	16	3.6	0
Undecylic	2.0	1	167	72	43.1	40.6
„	1.0–0.25	3	314	13	4.1	0
Lauric	5.0–0.5	4	369	14	3.8	0
Myristic	5.0–1.0	3	329	15	4.2	0
Undecenoic	2.0	1	110	60	54.6	52.6
„	1.0–0.5	2	210	12	5.7	1.75
„	0.25	1	156	5	3.2	0
Oleic	5.0	1	114	91	79.8	78.8
„	2.5	1	75	44	58.7	56.85
„	1.0	1	145	9	6.2	2.25
„	0.5	1	114	7	6.12	2.2
Stearic	5.0	1	86	11	12.8	9.1

The control figures for the percentage of eggs which did not hatch, irrespective of treatment (4.04 per cent.), is the mean of the results obtained from six lots of unsprayed eggs, four lots sprayed with 1 per cent. saponin solution and four lots sprayed with different concentrations

of benzene in 1 per cent. saponin solution—1676 eggs in all. The maximum range of variation in these controls was between 7.05 and 1.33 per cent. of eggs not hatching. This control figure is allowed for<sup>1</sup> in calculating the percentage of viable eggs killed, given in the last column of Table V. The figures show that none of the acids had any marked toxicity to these eggs at concentrations below 2 per cent. It is interesting however to compare the results obtained with the different acids at this concentration (2 per cent.) as set out in Diagram 7.

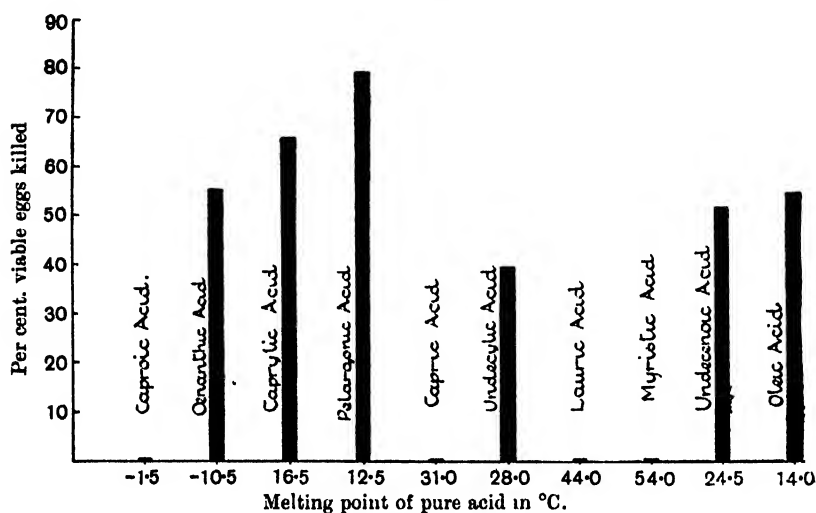


Diagram 7. Relative toxicities of fatty acids at a concentration of 2 per cent. to eggs of *Selenia tetralunaria*.

As the series is ascended from caproic to pelargonic acid, there is a rise in toxicity with increase in molecular weight; with the higher acids this no longer holds and only undecylic, undecenoic and oleic acids show any considerable toxicity. In attempting to account for these irregularities among the higher members of the series, it is perhaps suggestive that it is those acids with low melting points, *i.e.* those which are liquid at summer temperatures, which show toxicity. Penetration may take place more readily in the liquid state. Our figures would indicate undecylic acid to be rather exceptional in this respect. Our sample, however, under summer conditions, readily remained in a super-cooled state, and had a melting point slightly below that of the pure acid. It thus falls

<sup>1</sup> The average percentage which did not hatch in the controls (4.04 per cent.) is deducted from the percentage not hatched in each test and multiplied by  $\frac{100}{100 - 4.04}$ .



satisfactorily into line with the above suggestion. Observations on the stage of development reached by the majority of the unhatched eggs in each of these experiments give some additional evidence for this point of view. It was noted that in the case of undecenoic and oleic acids used at a concentration of 2 per cent., nearly all the unhatched eggs had failed to develop much beyond the point reached when they were sprayed, whereas with undecylic acid at the same concentration the majority of unhatched eggs contained almost fully developed larvae. It is therefore probable that undecenoic and oleic acids have greater penetrating powers than undecylic acid and that the difference between the effects of these acids is even greater than is indicated on the diagram. For a similar reason, the difference between the toxicities of oenanthic and pelargonic acids is probably understated in the diagram and table.

#### MOLECULAR WEIGHT IN RELATION TO INSECTICIDAL ACTION.

The correlation between the weight of the molecule and the toxicity of the fatty acid is not complete. The higher toxicity of formic acid as compared with acetic shows the importance of the constitution of the molecule; oleic acid is also more toxic than stearic although of almost the same molecular weight, and in many cases the methyl esters are less toxic than the acids of corresponding molecular weight (see Diagram 6). Moreover in passing up the series a point is reached where toxicity declines, thus myristic, palmitic and stearic acids show little or no toxic action. Either, therefore, there is some molecular weight value at which inhibition of toxicity takes place, or more probably certain physical properties come into play which act as limiting factors.

#### SOME PHYSICAL PROPERTIES CONSIDERED IN RELATION TO INSECTICIDAL ACTION.

In the following sections an attempt is made to see whether any marked correlation exists between toxicity to insects and certain physical properties of the fatty acids. Except in regard to compounds in the vapour phase, there is little published work on the subject of possible relationships between physical properties and insecticidal action. On the other hand, there is a considerable amount of information available as to the bearing of physical properties on narcotic action, rate of penetration through cell walls and other physiological phenomena. In certain respects these problems have some analogy or parallelism with the problem of insecticidal action and, without attempting to review

the extensive literature, we have made reference to certain facts and theories advanced in the study of these problems, which seem to have a bearing on our results.

### *Physical State.*

There is some evidence that compounds show a difference in the intensity of their toxic action to insects according to the physical state in which they are applied.

The data presented in a previous section on the toxicity of these acids to the eggs of *S. tetralunaria* suggest that the solid acids are less toxic than the liquid acids and that there is some correlation between toxicity and the melting point, *e.g.* capric acid (solid) is less toxic than pelargonic acid (a liquid) (see p. 346). It is not, however, clear that this consideration applies to the toxicity of these acids to aphids, for although such solid acids as myristic, palmitic and stearic show only a slight toxicity when compared with the liquid acids pelargonic, caprylic and oleic, yet capric acid (decylic), a solid, is more toxic than pelargonic (nonylic), which is a liquid at ordinary temperatures. It may be that the toxic effect of solid suspensoids is determined by the size of particle. The compounds of high melting point are somewhat difficult to obtain in a condition of very fine suspension, and there is a tendency for the particles to aggregate. As finely divided particles are more rapidly soluble than coarse ones, and as the lower melting point acids are more soluble in organic media than those of a higher melting point, the factor directly involved may be concerned with solubility in the cell membranes rather than with physical state. Consideration is given to solubility relationships in a later section.

### *Volatility.*

It has been shown by Moore<sup>(7)</sup> and by Tattersfield and Roberts<sup>(6)</sup> that there is a rough correlation between the boiling points, volatilities and vapour pressures of compounds and the toxicity of their vapours to insects. Within certain limits, the lower the volatility or vapour pressure, the greater the insecticidal action. Among similarly constituted compounds this is no doubt generally true, for it is reasonable to suppose that the more readily a vapour is adsorbed, the greater its insecticidal action, and "the adsorption on a carbon surface increases with the boiling point of the adsorbed gas<sup>1</sup>."

In the case of insecticides applied as sprays, the application of a

<sup>1</sup> Harkins, *Colloid Symposium Monograph*, II, 166.

similar conception is complicated by solubility and surface tension relationships. A highly volatile compound would presumably be less effective than a less volatile one, as it would evaporate more readily from the surfaces of the insect and have less time to act before being dissipated. This may account for the toxicities of methyl acetate, propionate and butyrate being apparently abnormally low. The relationship does not, however, always hold, since myristic and palmitic acids are less toxic than the more volatile acids, capric, pelargonic and caprylic; and further it is affected by considerations of chemical constitution. for example, the highly volatile methyl formate is more toxic than methyl butyrate.

#### *Dissociation Constants.*

The rate at which a compound penetrates would appear to be a determining factor in insecticidal action. The literature concerning the rate of penetration of the acids has grown to considerable dimensions, and although there are few instances where the experimental conditions correspond with ours, a brief résumé of some of the conclusions arrived at is of interest.

Adrian Brown<sup>(8)</sup> showed that the covering of the seeds of *Hordeum vulgare* shows a selective permeability to acids; it is not so readily penetrated by mineral as by organic acids. This property does not seem to be associated with the degree of dissociation, as relatively strong acids such as formic and chloracetic penetrate readily.

Loeb<sup>(9)</sup> found that mineral acids less readily induced membrane formation and were less rapidly toxic to the unfertilised eggs of sea-urchins than were the fatty acids. The greater the number of carbon atoms in the latter, the shorter the time necessary for membrane formation, a result intelligible on the assumption that those acids which diffuse most rapidly into the eggs call forth this response most readily. The conclusion drawn was that the acids diffuse into the cell only as undissociated molecules, particularly as certain of the sodium salts of the fatty acids gave negative results.

Harvey<sup>(10)</sup> found no correlation between the rate of penetration of the acids into the pigmented gonodial filaments of *Stechopus ananus* Jaeger, and the degree of ionisation; whereas Crozier<sup>(11)</sup>, from his experiments on the blue pigmented membranes of *Chronoderis zebra* Heilprim, considered ionisation to be a primary factor in determining cell permeability for acids, and that the degree of dissociation, if sufficiently great, practically controls the situation, but if sufficiently low,

as in the fatty acid series (formic acid excepted), some factor allied to capillary activity is dominant.

The dissociation constants for certain of the fatty acids are included in Table VI; and it is clear from this table that among the fatty acids dissociation constants cannot be correlated with insecticidal action. Formic acid, a fairly strong acid, is more toxic than acetic on the one hand, but less toxic than most of the higher fatty acids, and although the dissociation constants from acetic to nonylic (pelargonic) are all of the same order, there is nevertheless an increasing degree of toxicity.

### *Partition Coefficients.*

Meyer<sup>(12)</sup> in 1899 and Overton<sup>(13)</sup> in 1901 advanced the view that narcotic action was connected with the partition coefficients, *i.e.* the ratio of the solubility of the narcotic in oil or lipid to that in water. This theory has been subjected to a considerable amount of investigation and criticism. Bayliss<sup>(14)</sup> has pointed out that, although the theory shows how a narcotic obtains access to the cell, "it does nothing more in explanation of its action than to suggest that it is in some way exerted on the boundary membrane," the lipid constitution of which seems to have been widely accepted. It is generally considered that the theory is too specific to give an altogether satisfactory account of narcotic action. It is, in the main, an attempt to explain rate of penetration into cells. Nevertheless, this theory is of importance from our point of view, since readiness of penetration is probably a determining factor in insecticidal action. The results obtained by Phillipson<sup>(15)</sup> showed that the rates of passage of certain acids through collodion membranes impregnated with a complete etherial extract of muscle were in the following order: butyric > lactic > acetic > formic, but that the membrane was impermeable to mineral acids. Harvey (*loc. cit.*) considered that the penetration of the acids depended on several factors, *e.g.* lipid solubility, capillary activity, and an affinity for certain proteins of the cell surface. The results obtained by Loeb and by Crozier (*loc. cit.*) also indicated the importance of the Overton-Meyer theory. We therefore determined the partition coefficients as between olive oil and water of the fatty acids used in our experiments.

*Determination of the partition coefficients.* Olive oil of B.P. quality containing about 1 per cent. of free acid was used. A quantity of acid approximately equivalent to  $M/100$  was dissolved in the oil at 25–26° C., the exact concentration being determined by titration, allowing for the

free acid in the olive oil. In the case of myristic acid it was necessary to use *M*/200 on account of its low solubility. The oil solutions were then shaken up vigorously several times in stoppered cylinders with the same volume of water at 25° C. and allowed to stand in a constant temperature room at 25–26° C. for several weeks. The aqueous layer

Table VI.

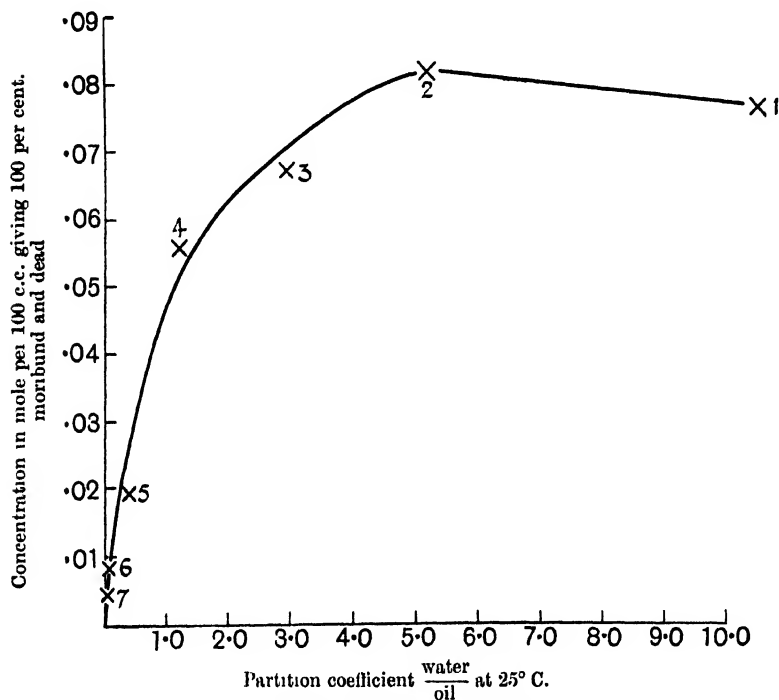
*Fatty Acids. Partition Coefficients for Water and Olive Oil, Dissociation Constants and Effect on Surface Tension of Water.*

No. of carbon atoms	Name	Mol. wt.	Oil layer Acid content before extraction gm./100 c.c.	A Oil layer Acid content after extraction gm./100 c.c.	B Aqueous layer Acid content after extraction gm./100 c.c.	Partition coefficients		Dissociation constants
						Oil/water A—B	Water/oil B—A	
1	Formic	46	0.398	0.0345	0.363	0.095	10.5	$2.14 \times 10^{-4}$
2	Acetic	60	0.591	0.006	0.495	0.19	5.15	(25° C.) $1.8 \times 10^{-5}$
3	Propionic	74	0.705	0.18	0.525	0.34	2.9	" $1.3 \times 10^{-5}$
4	Butyric	88	0.883	0.405	0.482	0.83	1.2	" $1.5 \times 10^{-5}$
5	Valerianic	102	1.018	0.738	0.28	2.64	0.38	" $1.6 \times 10^{-5}$
6	Caproic	116	1.19	1.06	0.13	8.15	0.12	" $1.4 \times 10^{-5}$
7	Oenanthic	130	1.27	1.234	0.036	34.27	0.029	" $1.4 \times 10^{-5}$
8	Caprylic	144	1.419	1.404	0.015	91.7	0.011	" $1.4 \times 10^{-5}$
9	Pelargonic	158	1.85	1.845	<0.0046	>401.2	<0.0025	" $1.1 \times 10^{-5}$
10	Capric	172.2	1.677	1.675	<0.0016	>1009	<0.001	—
11	Undecylic	186.2	1.71	1.697	<0.0129	Aq. solution turbid	—	—
12	Lauric	200.2	1.78	1.78	Trace	Very large	Very small	—
13	Tridecylic	214.2	1.92	1.918	<0.002	Aq. solution turbid	—	—
14	Myristic	228.3	0.975	0.975	Trace	Very large	Very small	—
15	Isocetic	242.3	Not available	—	—	—	—	—
16	Palmitic	256.3	1.7	1.7	—	Very large	Very small	—
17	Not available	—	—	—	—	—	—	—
18	Stearic	284.4	—	—	—	—	—	—
<i>Unsaturated acids:</i>								
11	Undecenoic	184.3	1.585	1.572	<0.0128	Aq. solution turbid	—	—
18	Oleic	282.3	2.63	2.63	Trace	Very large	Very small	—

The surface tensions at 25° C. of the saturated solutions in water of the following acids were in dynes/cm.:—Capric 32.61, undecylic 47.1, lauric 70.1, palmitic 71.2, oleic 71.3, water being taken as 71.8. (Traube's stalagmometer used.)

was carefully syphoned off in such a manner as not to draw over more than a trace of the oil layer, and allowed to stand for some time in corked tubes till clear, when a known quantity of the clear liquid was pipetted off and titrated with either *N*/50 or *N*/100 sodium hydroxide solution. In the case of undecylic, undecenoic and tridecylic acids, the aqueous layer maintained a persistent turbidity and the results could

not be taken. From the values obtained from the oil layers (before aqueous extraction) and those obtained from the final aqueous layers the partition coefficients could be determined. The determination of the amounts of the higher fatty acids dissolved in water can only be regarded as approximate<sup>1</sup>.



The acids are specified by the number of carbon atoms in the molecule ( $\times 1$  = formic acid).

Diagram 8. Showing relationship between toxicities of the fatty acids to *A. rumicis*

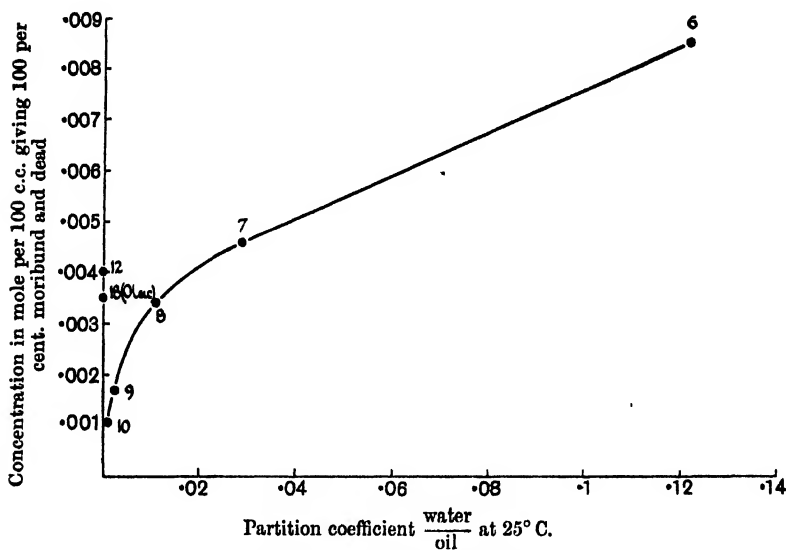
and their partition coefficients  $\frac{\text{water}}{\text{oil}}$ . (See also Diagram 9.)

The results are expressed in Table VI both as oil/water and water/oil solubility-ratios. In Diagrams 8 and 9 the water/oil solubility ratio is plotted against the lowest molar concentrations of the respective acids yielding 100 per cent. moribund and dead. This gives figures suitable for graphical representation.

The results are expressed in two diagrams of different scales, as the range of partition coefficients is so wide as to render obscure the results obtained for the higher acids if all are expressed on the same diagram.

<sup>1</sup> A surface tension method could not be applied under our conditions.

Diagram 8 gives a conspectus of the results for the acids formic (1 carbon atom) to oenanthic (7 carbon atoms). With the exception of formic acid, there is a steady rise in toxicity with a decrease of the partition coefficient (water/oil solubility ratio). Diagram 9 shows a similar close correlation from oenanthic to capric acid (10 carbon atoms). (Undecylic is omitted as the aqueous solution had a persistent turbidity rendering the determination unsatisfactory.) In the case of lauric acid<sup>1</sup> (12 carbon atoms) and of oleic acid (18 carbon atoms), there is a break



The acids are specified by the number of carbon atoms in the molecule  
(6 = hexylic, 18 = oleic).

Diagram 9. Showing relationship between toxicities of the fatty acids to *A. rumicis* and their partition coefficient  $\frac{\text{water}}{\text{oil}}$ .

in the correlation between toxicity and partition coefficient. They are both less toxic than would be anticipated on the assumption that the correlation held.

The coefficients, however, merely express the degree of separation of the acid between oil and water and are no measure of the absolute solubilities in either of the two media. As there is, in general, a decline in the solubilities of the solid acids in organic solvents with increase in molecular weight, this should probably be taken into account in considering any relationship between toxicity and the partition coefficient.

<sup>1</sup> The results of the spraying tests with lauric acid were less concordant than with the other acids; the average figures have been taken.

The differences in solubility in oil might explain the low toxicities of myristic, palmitic and stearic acids when compared with, say, decylic, and of stearic when compared with oleic, but they do not account for the low toxicity of oleic acid as compared with undecenoic, decylic or nonylic, oleic acid being miscible in all proportions with oil.

The Overton-Meyer theory does not distinguish clearly between the uses of oil and lipid in determining the partition coefficient, and the question arises whether a liquid fat like olive oil can be legitimately substituted for a solid or semi-solid lipid of the type of lecithin, which possesses hygroscopic qualities and complex solubility relationships<sup>1</sup>. A hydrophilic colloid of this type is capable of adsorbing certain water-soluble substances. The water-solubility relationships of the narcotic or toxic substances are brought into prominence by this consideration. The fatty acids have a group (carboxyl) that is definitely polar to water, a fact which has led to the modern theories of Langmuir and Harkins in regard to the orientation of these acids in the surface of their aqueous solutions. In addition they possess, in their hydrocarbon chain, a group definitely polar to products of the type of the organic solvents. Both of these properties are possibly of importance in determining passage through and adsorption by certain types of membranes and hence the toxicity of these compounds. The experiments of Adrian Brown (*loc. cit.*) suggested to him that the different rates of diffusion of the acids through the coverings of the barleycorn were due to some unrecognised peculiarity in which the molecules of the two classes of solutes were combined with the molecule of solvent water. It is perhaps suggestive that in our experiments saponification of the lower fatty acids, which materially increases their solubility in water, decreases toxicity; this effect declines as the length of the hydrocarbon chain increases until in the case of oleic, the ammonium and sodium soaps are, if anything, more toxic than the acid.

It may therefore be concluded that despite the fairly close correlation between the toxicities of many of the acids and the partition coefficients, the application to insecticidal action of the Overton-Meyer theory of narcotic action does not afford a complete explanation of our results. It cannot be applied without consideration of chemical constitution<sup>2</sup> and other physical properties. Among the fatty acids the correlation is,

<sup>1</sup> Most of the work since Overton and Meyer indicates that the cell surface is more complex than is represented on the lipid theory.

<sup>2</sup> *E.g.* methylation of the carboxyl group which presumably decreases the values of the partition coefficients water/oil results in a general decline in toxic properties.



however, suggestively close and there is obviously need for further work on the relationships existing between solubility, penetration and toxic action.

### *Surface Tension.*

It has been realised for some considerable time that surface tension relationships are of significance, and indeed are probably one of the determining factors in the problems of permeability, penetration and narcotic action. In a long series of papers since 1904, Traube<sup>(16)</sup> has stressed the importance of this physical property with respect to osmosis and penetration. He has applied his "cohesion pressure"<sup>1</sup> (*haftdruck*) theory to problems of narcosis, disinfection and the physiological action of drugs and poisons. The Overton-Meyer theory of narcosis is criticised on the grounds that lipoid-free cells can be subjected to the action of narcotics<sup>(17)</sup>, an objection not applicable to his theory, which is largely based upon the view that compounds with the property of lowering surface tension will cause a change in the physical state of the cell contents, with a consequent retardation of certain metabolic processes such as oxidation. Czapek<sup>(18)</sup> has shown that surface tension effects are of considerable importance in determining the penetration of the fatty alcohols and other organic chemicals into living cells. Working with several of the higher plants, he showed that penetration took place when the aqueous solutions of the alcohols attained a critical value of about 0.68–0.69 of the surface tension of water.

It was realised by both Harvey and Crozier (*loc. cit.*) that capillary activity might be one of the factors intimately bearing on the problem of the penetration of acids. The surface tensions of the aqueous solutions of fatty acids from formic to decylic (with the exception of octylic) have been thoroughly worked out by Harkins, Clark and King, and very complete data have been published by King<sup>(19)</sup>. King shows that from formic to decylic the relative lowering of the surface tension of water increases with the molecular weight of the acid; thus a solution of 18.6 moles of formic acid per litre causes a reduction at 20° C. from 72.8 to 41.2 dynes/cm., whereas a reduction to approximately the same figure is produced by decylic acid at a concentration of only .0002 mole per litre. There is on the whole a similarity between the adsorption curves of King and our curves relating toxicity with concentration as shown in Diagrams 1 and 2. The data given by King (*loc. cit.*) do not go beyond decylic acid, and his experiments show that the true surface

<sup>1</sup> The more a substance lowers the surface tension of a solvent, the smaller its cohesion pressure when dissolved in it.

tension value of the solution of this acid is not reached in the drop-weight method until a period of 30 minutes has been allowed for the solution to come into equilibrium with its surface. Frumkin<sup>(20)</sup> estimated that solutions of lauric acid require several hours. A few preliminary determinations of the surface tension of the aqueous solution of the higher acids have been made by us, but much further work is required before deductions can be drawn in regard to the relationship between surface activity and the insecticidal action of these compounds. Using a Traube stalagmometer, with which only a short time can be allowed for surface equilibrium to be established, we observed that from lauric acid upwards, the surface tensions of the saturated solutions approximated to that of pure water. It may be suggestive that toxicity among the acids begins to decline with lauric acid, but this decline could not be correlated in any simple way with the surface tension of the aqueous solutions of the respective acids. The lowered capillary activity under these conditions is due to the solubility of these acids being very low and the molecules sparsely distributed in the solution. This leads to the question already raised (p. 355) as to whether the toxicity of these acids is not in some way bound up with water-solubility relationships.

#### SUMMARY.

1. The toxicities to *Aphis rumicis* L. of the fatty acids from formic to stearic and of the sodium and ammonium salts and methyl esters, applied as spray fluids, have been quantitatively determined. Two unsaturated acids, undecenoic and oleic, are included.

2. There is a rise in toxicity of the acids with increase of molecular weight as the series is ascended from acetic to undecylic acid. Formic acid is exceptional. Beyond undecylic acid, there is a fall in toxicity, and acids higher in the series than tridecylic show only slight toxic action.

3. The sodium salts of the fatty acids are in most cases much less toxic than the corresponding acids, though the difference is less marked with the higher acids. Oleic acid and sodium oleate are of the same order of toxicity.

4. The ammonium salts are also generally less toxic than the corresponding acids, but the differences are much less than in the case of the sodium salts. With some of the higher acids, *e.g.* myristic and oleic, neutralisation with ammonia increases toxicity. The relatively high toxicity of the ammonium salts may be due, at least partly, to liberation, by hydrolysis, of free fatty acid in a very finely divided state.

5. Methylation of the fatty acids reduces toxicity; all the methyl esters are less toxic than the acids or ammonium salts.

6. Both the ammonium salts and the methyl esters show, like the acids themselves, increase of toxicity with increase of molecular weight up to a certain point. The formates are exceptional.

7. The fatty acids do not show marked toxicity to the eggs of *Selenia tetralunaria* Hüfn. at concentrations below 2 per cent.

8. Possible relationships between certain physical properties (physical state, volatility, dissociation constants, partition coefficients and surface tension) of the fatty acids and their insecticidal action are discussed.

9. Determination of partition coefficients as between olive oil and water and comparison of the figures with the relative toxicities show a steady rise in toxicity with a decrease in the partition coefficients (water/oil) from acetic to capric acid. Formic acid is again exceptional. With lauric and oleic acids there is a break in the correlation. The bearing of the solubility relationships of the acids on these results is considered.

#### LITERATURE CITED.

- (1) SIEGLER, E. H. and POPENOE, C. H. (1924). *Journ. Agric. Res.* xxix, 259.
- (2) ——— (1925). *Journ. Econ. Ent.* xviii, 292.
- (3) TATTERSFIELD, F. and MORRIS, H. M. (1924). *Bull. Ent. Res.* xiv, 223.
- (4) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* xii, 61.
- (5) ——— (1925). *Ann. App. Biol.* xii, 218.
- (6) TATTERSFIELD, F. and ROBERTS, A. W. R. (1920). *Journ. Agric. Sci.* x, 199.
- (7) MOORE, W. (1917). *Journ. Agric. Res.* x, 365.
- (8) BROWN, A. J. (1907). *Ann. Bot.* xxi, 79.
- (1909). *Proc. Roy. Soc. B*, lxxxi, 82.
- (9) LOEB, J. (1913). *Artificial Parthenogenesis*. (Univ. of Chicago.) Chap. xiv, 133.
- (10) HARVEY, E. N. *Internat. Zeit. phys.-chem. Biol.* i, 462. (*Science* (1914), xxxix, 947.)
- (11) CROZIER, W. J. (1916). *Journ. Biol. Chem.* xxiv, 255.
- (12) MEYER, H. H. (1899). *Arch. Exp. Path.* xlii, 109.
- (13) OVERTON, E. (1901). *Studien über die Narkose*. Jena.
- (14) BAYLISS, W. M. (1924). *Principles of General Physiology*, p. 140.
- (15) PHILLIPSON, M. and HANNEVART, G. (1920). *Compt. Rend. Soc. de Biol.* lxxxiii, 1570.
- (16) TRAUBE, I. (1904). *Pflüg. Archiv Physiol.* cv, 541.
- TRAUBE, I. and SOMOGYI, R. (1921). *Biochem. Zeit.* cxx, 90.
- (17) TRAUBE, I. (1913). *Pflüg. Archiv Physiol.* clii, 276.
- (18) ČZAPEK, F. (1910). *Ber. Bot. Ges.* xxviii, 480.
- (19) KING, H. H. (1922). *Techn. Bull.* 9, *Kansas Agric. Exp. Sta.*
- (20) FRUMKIN, A. (1925). *Z. physik. Chem.* cxvi, 466.

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# INVESTIGATIONS ON THE CONTROL OF WIREWORMS

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(With Plates XXIV and XXV and 2 Text-figures.)

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## I. THE WIREWORM PROBLEM.

### *Historical.*

WIREWORMS cause considerable annual losses amongst agricultural and horticultural crops, and though these losses are especially severe for the first three or four years after land has been broken up from grass or ley, more or less injury occurs year by year when the wireworm content has reached its normal level. In general farming areas such crops as turnips, swedes, potatoes, barley and oats are particularly susceptible

to attack, while in horticultural areas tomatoes and chrysanthemums frequently suffer severely.

During the Great War the demand for increased food production led to the breaking up of grassland and concentrated the attention of farmers and others on injurious soil insects. Applications for advice to the Board of Agriculture and the Advisory Centres indicated that the wireworm was one of the most troublesome and common of soil pests and the wireworm problem was studied by several research workers in an effort to obtain fuller information and if possible discover suitable methods of control.

The susceptibility of various kinds of garden crops to injury by wireworm was studied by A. H. Lees at the Long Ashton Research Station<sup>(8)</sup>. Lees studied the vegetable crops grown on land within two years of breaking up and grouped them into classes according to the degree of susceptibility to wireworm injury. Thus plants like onions, leeks, celery and lettuce which were attacked at the fleshy collar, and killed, dwarfed or caused to go to seed prematurely were classed as "very susceptible." Plants like runner beans, dwarf beans and peas were "rather susceptible," being dwarfed but not usually killed. The cabbage tribe and tomatoes were "slightly susceptible," but the former ran less risk as the plants were fairly hard about the collar at the time of planting out. In a fourth group, wireworm injury did not endanger the life of the plants: thus with potatoes the injury was confined to the tubers (Pl. XXIV, fig. 1), considerably decreasing their market value but the collar of the plant escaped; carrots also would belong to this group. The practical application of these findings was that crops on new land could be selected with due regard to the probability of wireworm attack, and highly susceptible crops avoided.

A. Roebuck<sup>(19)</sup>, Harper Adams Agricultural College, studied the wireworm content of grass fields and newly broken up fields, keeping the latter under observation each year until they passed into normal cultivation. He found by calculation that certain grass fields near Newport, Salop, contained from 215,000 to 510,000 wireworms per acre, the majority located within the surface two inches of soil<sup>(1)</sup>. Roebuck also studied cultural treatment for the control of wireworms, noting that cultivation and systems of cropping exercised considerable influence on the pest.

G. H. Ford<sup>(1)</sup>, Manchester University, made a study of the larval and pupal stages of *Agriotes obscurus* L., the larvae of which are the most common wireworm in Cheshire, North Staffordshire and South Lancashire.

Probably by far the most important investigation was that initiated at the Rothamsted Experimental Station in 1916 by A. W. Rymer Roberts<sup>(16)</sup>, who studied in great detail the life history and structure of wireworms of the genus *Agriotes* and made some observations on those of *Athous haemorrhoidalis* F., rearing the former species from egg to adult which took from August 1916 to the middle of 1921, and determining that under English conditions the length of life in the larval stage may be of six years' duration.

Much was added to our knowledge of wireworms by the research carried out from 1915 to 1920 but no definite means of controlling the pest was developed, though some attention was paid to control by cultural means by Harper Gray<sup>(12)</sup> and others, and local trials with soil insecticides, chiefly of a proprietary nature, were carried out. Tattersfield and Roberts<sup>(21)</sup>, however, studied the toxicity of organic compounds to wireworms and found that compounds with irritating vapours have usually high toxic values, *e.g.* allyl isothiocyanate, chloropicrin benzyl chloride, while chemically inert compounds boiling above 170° C. are generally uncertain in their poisonous effect on wireworms after an exposure of 1000 minutes at 15° C.

Post-war conditions, far from stimulating work on the control of soil pests, have adversely affected it and the general tendency has been for farmers to lay more land down to grass. In areas where intensive culture is followed and where industrial areas have provided stimulus for market growing, land is, however, constantly being broken up from grass or ley. Here the wireworm problem is still serious and the demand for effective control measures urgent; for potatoes, cruciferous crops and beans in intensive areas; strawberries in fruit-growing areas; peonies, pyrethrums, asters, chrysanthemums and certain other flowers in market growing areas; and tomatoes and other crops under glass, are all liable to severe injury by the depredations of this pest.

In the autumn of 1925 investigations on the control of wireworms (10), (11) were commenced, and these have now reached a stage when definite recommendations can be made. The following is a complete account of the work.

The writers acknowledge their indebtedness for suggestions and advice to Mr J. C. F. Fryer, M.A., Director, Ministry of Agriculture Pathological Laboratory, and for assistance to Mr W. G. Kent, N.D.H., Horticultural Superintendent, Isle of Ely. Thanks are also due to Mr Frank Waite of Boston, Mr Ivor Mard of Hatfield and Mr Yates

Christie of Worthing, for their kindness in placing land or glasshouses at our disposal for the investigation.

(2) *Observations on wireworm attacks.*

Since wireworms are most troublesome when grassland is broken up the general practice is to grow special crops for two or three years before adopting the normal rotations followed in the district. Thus oats, potatoes, and mustard are often selected during the first few years. From the writers' observations oats appear to be less susceptible to wireworm attack than either wheat or barley; they tiller freely and, even when attack is serious, make sufficient tillers to carry a crop and yield fairly satisfactory returns. Potatoes do well on new land and after a crop of potatoes there is generally a marked diminution in the wireworm content of the soil, presumably owing to large numbers being removed with the crop. There is some risk of the seed tubers being attacked but when land is newly broken up there is usually sufficient undecayed turf in the soil to provide food for the wireworms for some time. In Lincolnshire it was frequently noticed that early potatoes on newly broken up land escaped attack by wireworms while late crops under similar conditions were often severely infested, probably owing to exhaustion of other food. Mustard usually does well on new land and suffers very little from the attacks of soil insects, hence it is commonly taken as a first crop in Lincolnshire and followed by potatoes the next year.

In fruit-growing areas where land is broken up for strawberry culture it is customary to take one crop, usually potatoes, before planting out strawberries. For the first year or two after planting, however, some proportion, often a high one, is lost through wireworm depredations. The wireworms tunnel into the young crowns and cause death to the growing point, or gnaw at the young roots so weakening the plants that many finally perish.

Where flower culture is followed losses often occur especially among cuttings and young plants. In chrysanthemum-growing districts—West Sussex, Hampshire, Cambridge and Norfolk—the usual practice is to plant rooted cuttings out in the field. The wireworms tunnel up the stems of these causing considerable loss. In the autumn the surviving plants are taken into the glasshouses, with the result that wireworms may be introduced amongst the roots, and their attack on subsequent crops like tomatoes often results in the loss of from a quarter to a third of the plants. Asters and stocks pricked out as seedlings also suffer from wireworm attacks; and pyrethrums, which otherwise do

exceptionally well on new land, have often to be replanted. In May 1926 examination of 18 pyrethrum plants on a flower farm in Cambridgeshire revealed a total of 18 wireworms present; 10 of the plants were free from the pest but 4 of the remaining plants had 3 wireworms at the roots of each, 2 had 2 wireworms each and 2 had 1 each. Carnations too, frequently suffer from wireworm depredations and here again the characteristic tunnelling up the stem is apparent.

Tomatoes in glasshouses often suffer severely from attack by wireworms. In a case which came to the writers' notice records showed that out of 2196 plants 580 were destroyed by wireworms, and several growers state that they have lost more than half their plants owing to wireworm attacks. In a block of glasshouses near Worthing where tomatoes and chrysanthemums were grown alternately, 10 representative soil samples, 6 in. square and 18 in. deep, taken at the end of the fourth tomato season, yielded 9 wireworms: by calculation, a wireworm content of 156,816 per acre. Careful examination of the attacked plants show that the wireworms most commonly enter the stems just below the surface-feeding adventitious roots and tunnel upwards from 5 to 8 in. (Pl. XXIV, fig. 2), causing the plant to become dwarfed, greyish in colour and wilted, finally inducing death. In cases of high infestation 5 or 6 wireworms per stem may be found, the attack becoming apparent within a day or two after setting out the plants.

An interesting case of wireworm attack on vines was observed in Middlesex. Young vines were planted in October 1925 in a properly prepared vine border to which a quantity of new turf had been added. The vines did not make good headway and eventually the trouble was traced to wireworms which were destroying the roots as fast as they were being produced.

During the winter of 1924-5 the writers examined numbers of swede seed plants which were suffering from various bacterial rots and in many cases the plants had been first attacked by wireworms, often several wireworms occurring in characteristic tunnels in the plants. Mangold plants set out for seed were found attacked in a similar manner, especially during mild periods in the winter months.

The nature of wireworm attack on ordinary farm crops is so well known as to need no special description. The wide range of plants in both general agriculture and commercial horticulture which may suffer from the depredations of this pest bears out the truth of Curtis's statement<sup>(3)</sup> that "in the wireworm we have an example of a larva which



may be termed omnivorous as far as regards the productions of the field and garden."

The wireworms occurring most commonly during the writers' investigations in the County of Holland, Lincolnshire, were the larvae of *Agriotes obscurus* L. Odd specimens of the larvae of *Athous haemorrhoidalis* F. occurred occasionally at the roots of strawberries and crops grown in bays between fruit trees. The larvae of *Dolopius marginatus* L. also occurred in Lincolnshire and were often taken during 1924-5 feeding at the roots of cabbages and cauliflowers. The wireworms met with in glasshouses were the larvae of *Agriotes obscurus* L. and *A. sputator* L. These two species were found in numbers in two localities in Hertfordshire and in West Sussex.

### (3) *Feeding habits of wireworms in relation to weeds.*

In order to obtain information on the natural food preferences of wireworms, a survey of the weeds on an arable field in Lincolnshire known to be infested with wireworms was made in the autumn of 1925. Wherever possible 100 specimens of each species of weed were examined but in many cases it was impossible to find that number. Each plant with its roots and the surrounding soil was carefully lifted and shaken and the number of wireworms found was recorded. The figures are given in the following table:

Table I.

Species	No. examined	Wireworms
<i>Agropyrum repens</i>	100	56
<i>Taraxacum officinale</i>	100	20
<i>Senecio vulgaris</i>	100	15
<i>Plantago lanceolata</i>	100	20
<i>Poa annua</i>	100	7
<i>Stellaria media</i>	100	4
<i>Bromus</i> sp.	100	3
<i>Chenopodium album</i>	100	1
<i>Urtica urens</i>	54	0
<i>Sonchus</i> spp.	34	3
<i>Cardamine hirsuta</i>	24	2
<i>Achillea millefolium</i>	14	21
Miscellaneous grass seedlings	11	2
<i>Dactylus glomerata</i>	10	1
<i>Urtica dioica</i>	7	0
<i>Rumex acetosa</i>	5	0
<i>Polygonum persicaria</i>	4	0
<i>Veronica chamaedrys</i>	3	0
<i>Potentilla anserina</i>	1	0

The observations indicated that the relation between the wireworms and the plant was influenced largely by the character of the underground portion of the plant. The moist and fibrous nature of the underground portions of yarrow and couch grass proved particularly attractive; the tap roots of dandelions were less so; and dry, fibrous roots like those of chickweed, goutweed and nettle seemed to be avoided. The specimens of groundsel were large and had well-developed roots and those of *Plantago* also had good compact root systems. In addition to the weeds, volunteer potatoes and small cabbage plants were also examined with interesting results; 100 small potato plants yielded 114 wireworms and 100 cabbage plants 31 wireworms.

#### (4) *Seasonal movements of wireworms.*

Wireworms may be found within a few inches of the surface of the soil at almost any season of the year, but there are certain well-marked periods when they are particularly prevalent. In two plots at Rothamsted, Herts. examined periodically from February 1920 to January 1921, Morris<sup>(13)</sup> found wireworms each month, and of the total wireworms taken 32.2 and 32.6 per cent. were located in the soil from 5 to 7 in. deep. In Aberystwyth area in 1920-21 M. Thompson<sup>(23)</sup>, sampling soil from June to the following May, found wireworms within the top 3 in. of soil each month except August, the highest number for a sample occurring in April. Hammond<sup>(5)</sup> arrived indirectly at the seasonal prevalence of wireworms by examination of the crops of 10 starlings each month for a year, the maximum numbers of wireworms occurring in the crops in April, and the minimum during the summer months. In like manner Theobald and McGowan<sup>(22)</sup> found that wireworms occurred in greatest numbers in March and October to December.

Observations on the winter movements of wireworms in the soil were taken by the writers from October 1925 to March 1926. Soil samples 6 in. square were examined in three layers: the surface 3 in., the section 3-6 in. deep and the section 6-9 in. deep (vide Table 11).

Expressed graphically (Fig. 1) the figures show high peaks in October and March for the top 3 in. of soil, with a depression between them corresponding to a high peak in the 6-9 in. which reached its apex in December and fell off from January to March. In other words there appeared to be a steady migration of wireworms down deeper into the soil in October, followed by fluctuations, probably in response to local conditions of temperature and moisture, and a steady upward movement from January to March. From observations taken in the

Table II.

Date	No. of samples containing wireworms	Wireworms present in		
		Surface 3 in.	3-6 in.	6-9 in.
Oct. 4 and 5	17	19	6	9
" 15	5	6	4	0
" 19	8	6	7	1
" 29	10	6	7	1
Nov. 6	10	5	8	3
" 14	10	0	7	4
" 20	10	0	5	9
" 24	10	2	4	5
" 27	3	0	1	2
Dec. 4	10	0	4	11
" 19	10	2	6	7
" 28	10	1	10	6
Jan. 2	10	0	5	8
" 9	10	1	11	8
" 15	10	0	5	8
" 23	10	1	4	7
" 30	10	1	6	7
Feb. 6	10	1	8	4
" 13	10	1	6	4
" 20	10	3	7	4
" 27	10	3	5	4
Mar. 6	10	4	2	4
" 13	10	7	7	3
" 20	10	11	6	4
" 27	10	7	5	2

field there seems to be another migration of the wireworms downwards in summer, again probably in response to temperature and moisture conditions. Thus crops which have been seriously checked by wireworm attack often progress satisfactorily in June and July, and during the period from June to August it is often difficult to find wireworms except at some depth in the soil. In a series of soil samples taken in a glasshouse at Worthing in September the majority of wireworms were located at from 12 to 15 in. deep and some occurred 15 to 18 in. deep. The temperature at 15 in. deep was 58° F. while at the surface the soil temperature was 60° F. and the top few inches of soil were very dry, conditions likely to occur in the open at midsummer. With the advent of autumn these soil conditions change and an upward migration of wireworms is again apparent.

Seasonal movements of wireworms have an important bearing on control measures, for operations carried out in mid-summer or mid-winter

would be less likely to yield successful results than operations carried out in spring or autumn when the maximum number of wireworms occur in the surface soil. Moreover in mid-summer the land is usually under crops and cannot be disturbed while in mid-winter the wet and frozen

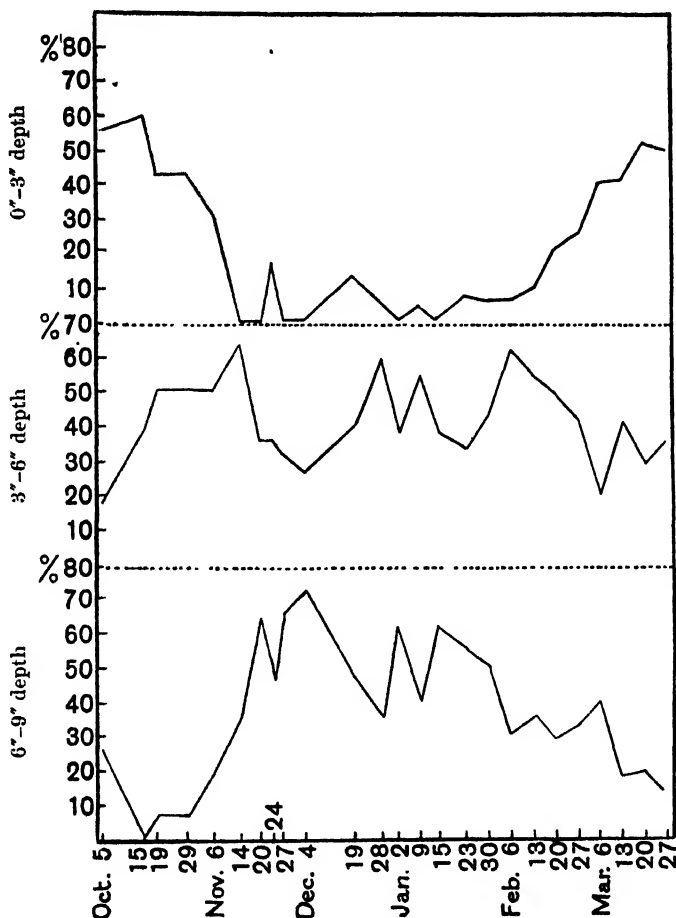


Fig. 1. Graph showing seasonal movements of wireworms from data obtained in Lincolnshire, October 1925-March 1926.

condition of the soil would render cultivation impossible; but in spring and autumn, prior to cropping and after the removal of the crops, routine cultivation can be made use of in connection with the control of wireworms.

## II. BAITING AS A MEANS OF ASSEMBLING WIREWORMS.

Ormerod<sup>(11)</sup> and Miall<sup>(9)</sup> discuss the use of rape dust and rape cake for attracting wireworms and the former mentions that when a dressing of these substances is applied to a crop the wireworms leave the crop to feed upon the rape. Potatoes are mentioned by Curtis<sup>(3)</sup> as a suitable bait for attracting wireworms; and Wardle and Buckle<sup>(21)</sup> give a brief account of field work in California where potato baits, planted in lines down the field and periodically examined, resulted in the trapping of as many as 4000 wireworms per acre. Since then (1913) little was done on the use of baits until the discovery of substances toxic to wireworms, but too expensive for widespread use under general farming conditions, drew the attention of American entomologists to the possibility of using bait as a means of assembling wireworms into well defined areas when treatment could be applied at a greatly reduced cost. This was referred to in 1924 by Campbell<sup>(2)</sup>, who suggested that split beans and rice bran might be used for this purpose, and in October 1925 Spuler<sup>(20)</sup> published an account of work done in Washington on the use of such baits as bran, potatoes, carrots, peas, beans and maize. He arrived at the conclusion that germinating seeds attract more wireworms than any other bait; moreover, seeds are easily planted and the bait rows can be readily traced.

(1) *Field Experiments 1925.*

In October 1925 the writers commenced investigations at Boston, Lincolnshire, to test the efficiency of baiting as a means of assembling wireworms and to compare the merits of various substances for use as bait. The experiments were carried out in a field lent for the purpose by F. Waite, Esq., of Boston. The field had been broken up early in the spring of 1925 and a crop of early potatoes grown. Later cabbages had been drilled but these were a failure, so that save for a few cabbage plants and odd weeds of the species listed on p. 364 the land was fallow. The soil was typical Lincolnshire silt with a considerable amount of more or less undecayed turf, and here and there were pockets of soil of a heavier nature. A portion of the field about 4 acres in extent was selected for the trials and the soil sampled for wireworms, 83 samples each 6 in. square and 9 in. deep yielding 81 wireworms, or approximately 170,000 per acre, the majority located within the top 3 in. of soil.

The land was first cleared of weeds and lightly worked with a hoe; then from October 5th to 12th 21 rows, each 10 yd. long and 5 ft. apart,

were set out with baits at a depth varying from 2-4 in. The baits, selected according to cheapness and availability, included chopped potatoes, oats, bran, wheat, peas and beans. They were examined after a period of 14 days, the whole row, 6 in. wide and 6 in. deep, being taken out and sifted, and the assembled wireworms removed and their numbers recorded. The following table gives a list of the baits used, indicates the order of the bait rows and shows the results obtained.

Table III.

Row No.	Bait	Depth of planting in.	Wireworms attracted
1	Potatoes	4	41
2	Oats	2-3	89
3	Wheat	2-3	88
4	Potatoes	3	24
5	Beans	2-3	51
6	Peas	2-3	33
7	Potatoes	2	15
8	Grass	4	34
9	Oats	2-3	32
10	Wheat	2-3	46
11	Grass	3	55
12	Beans	2-3	41
13	Peas	2-3	42
14	Grass	2	37
15	Bran	4	64
16	Oats	2-3	38
17	Wheat	2-3	47
18	Bran	3	52
19	Beans	2-3	34
20	Peas	2-3	62
21	Bran	2	47

The results obtained from this experiment indicated that wireworms could be readily attracted to baits. In the total area baited 972 wireworms were assembled, by calculation about 13,000 per acre, in 14 days from the setting of the bait. A comparison of the total number of wireworms attracted to each bait is interesting; wheat attracted 181 wireworms, bran 163, oats 159, peas 137, beans 126, grass 126, and potatoes 80.

The influence of depth of baiting on the assembling of wireworms was shown by the preceding experiment. A comparison of similar baits set at varying depths is given in Table IV.

Table IV.

Bait	Depth in.	No. of wireworms	Total
Bran	2	47	99
Grass	2	37	
Potatoes	2	15	
Bran	3	52	131
Grass	3	55	
Potatoes	3	24	
Bran	4	64	139
Grass	4	34	
Potatoes	4	41	

This indicated that bait planted at a depth of 3-4 in. was more efficient than that planted 2 in. deep.

The next experiment was designed to compare the rates at which wireworms were attracted to various baits. With the exception of grass, which was difficult to handle, baits similar to those used in the previous experiment were set 2-3 in. deep in rows 6 ft. apart and 20 yd. long, the series being planted in duplicate. Each day a sample 1 yd. long, 6 in. wide and 4 in. deep was sifted out from each row, the first examination occurring 24 hours after the setting of the baits. Table V and Fig. 2 give the results obtained over a period of 24 days.

This experiment indicated that bran and wheat attract wireworms most speedily, and that oats, peas, beans and potatoes increase in attractiveness from 6 to 12 days after setting. The attractiveness of bran for the wireworms decreased after about 12 days; peas and beans showed a fairly steady attraction during the last 18 days, and potatoes showed rather poor attraction throughout the entire period. The outstanding results were obtained from wheat and oats, which showed considerable attraction during the last 18 days of the period, assembling large numbers of wireworms during the final week of the experiment. The data obtained from this experiment suggested that the time available for baiting would have some bearing on the bait chosen. Where only a short time could be spared bran or wheat or a mixture of both might be set, or if a longer period were available oats might be selected as bait.

In order to secure information as to the distance over which baits would prove attractive, two experiments were carried out; the first with field beans set in rows at varying distances up to 6 ft. apart, and the second with wheat drilled in rows at varying distances up to 10 ft. apart. The beans were sown on October 7th and examined 14 days later, and the wheat was drilled on October 25th and examined on November 14th. The rows were sifted out in the usual way and the number of wireworms

Table V.

*Attractiveness of Wireworm Baits.*

Bait	Date examined October												Total 1st 12 days	Date examined October                      November												Total 2nd 12 days	Total for period
	Wireworms attracted													October						November							
	13	14	15	16	17	19	20	21	22	23	24	26		27	28	29	30	31	2	3	4	5					
	Wireworms attracted													Wireworms attracted													
Bran	.	4	3	2	6	6	6	10	9	9	11	66	2	5	4	1	2	0	1	0	0	1	16	82			
Oats	.	6	1	1	2	2	3	2	6	6	2	31	10	1	15	11	5	22	14	5	1	2	95	126			
Wheat	.	7	1	0	2	9	7	10	12	6	5	59	3	5	1	2	2	9	8	6	8	11	55	114			
Peas	.	1	2	3	2	1	4	4	12	7	4	40	4	2	2	3	2	7	2	14	3	4	43	83			
Beans	.	1	2	1	1	7	1	1	4	5	3	29	1	0	1	4	0	9	4	6	5	9	39	68			
Potatoes	.	1	0	1	0	1	2	6	16	19	7	44	2	2	0	0	2	4	4	4	3	4	25	69			
Bran	.	4	2	2	12	9	5	17	7	10	3	71	4	0	2	3	3	2	2	0	1	2	19	90			
Oats	.	2	2	1	2	11	4	3	9	8	5	47	4	1	8	8	3	20	8	14	12	17	95	142			
Wheat	.	2	5	4	3	8	5	7	6	15	8	63	14	6	2	7	7	17	12	17	12	8	102	165			
Peas	.	6	1	2	1	2	3	8	13	5	6	50	6	4	7	6	6	2	9	6	4	7	57	107			
Beans	.	2	1	3	6	6	2	13	6	7	5	51	6	3	2	5	5	3	0	4	0	10	38	89			
Potatoes	.	3	3	1	1	6	1	4	5	13	3	46	6	2	6	2	5	8	2	2	1	3	37	83			

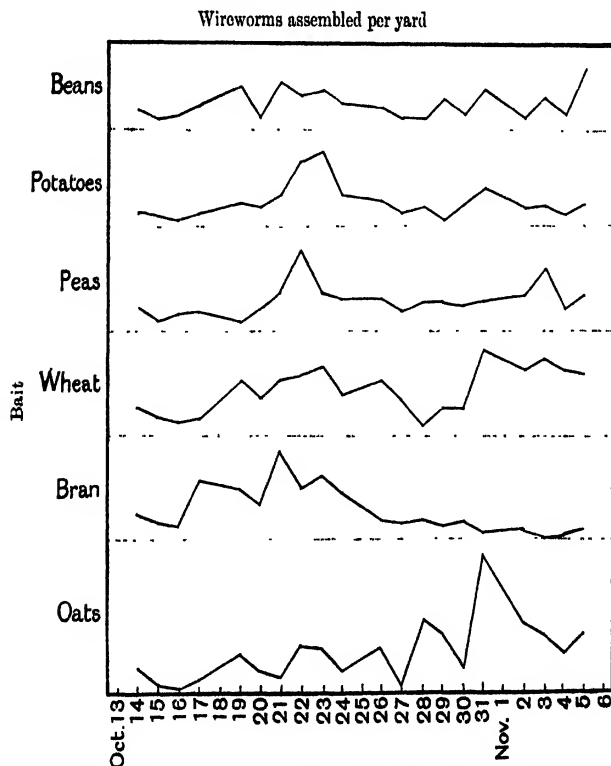


Fig. 2. Graph showing attractiveness of bait as obtained from trials in Lincolnshire, October and November 1925.



recorded. Since sifting was impossible in the time available the interspaces were carefully dug over, therefore the figures for wireworms in the interspaces and the percentage attraction cannot be regarded as strictly accurate though they give a good indication of the influence of the distance between the baits. In the case of the wheat, cold and frosty weather occurred during the period of baiting and this may have driven the wireworms deeper into the soil especially in the wider interspaces where no food was available. The results of this experiment, given below, indicate that it is undesirable to set bait rows further apart than 4 ft.; and in subsequent experiments the bait was usually drilled in rows 3 ft. apart, since the ordinary corn drill could be easily arranged to drill two rows at this distance apart.

Table VI.

Bait	Row No.	Distance apart ft.	Wireworms attracted	Wireworms in interspace	Percentage attracted
Beans	1	—	26	—	—
	2	2	17	3	85
	3	3	15	4	79
	4	4	12	6	67
	5	5	14	18	44
	6	6	10	21	32
Wheat	1	—	11	—	—
	2	2	15	1	94
	3	3	6	1	86
	4	4	10	1	91
	5	5	8	4	67
	6	6	11	8	58
	7	7	12	2	86
	8	8	12	7	63
	9	9	27	4	87
	10	10	25	4	86

On November 12th the area unoccupied by these various experiments, about  $3\frac{1}{2}$  acres in extent, was well harrowed and drilled with wheat to test on a field scale the value of baiting in assembling the wireworms. The corn drill used was the usual type of horse-drawn machine, about 6 ft. 6 in. wide, and the intermediate spouts were cut out to leave two running about 3 ft. 3 in. apart. The wheat was planted about  $2\frac{1}{2}$  in. deep.

A sudden change in the weather at this time resulted in almost continuous frost and some snow for a considerable time; and periodic examinations showed that only a few wireworms visited the bait. The trial was finally abandoned because in the spring, before the upward

movement of the wireworms in any numbers was apparent, it was necessary to prepare the field for the 1926 cropping.

Several lessons were learnt from these experiments. Potatoes, which are often advocated for trapping wireworms, proved much less attractive than other baits, especially germinating seeds. Bran, too, was not attractive for a long period and after about 10–12 days began to develop mould, which probably rendered it unpalatable to the wireworms. Peas, beans and large pieces of chopped potato were found to be unsatisfactory where baiting was to be followed by the use of an insecticide, for wireworms, particularly small ones, could be found tunnelling inside, where they were probably beyond the reach of the insecticide. The use of seeds which would germinate had a marked advantage over bait which would not grow, for after the period necessary to allow for the wireworms assembling, the bait rows could be easily traced by the green shoots which appeared above the ground. Since the wireworms attacked the young plants at the base of the plumule after the food stored in the grain was exhausted, where the pest was very numerous at the bait only a few green blades could be seen.

## (2) *Field Experiments 1926.*

To test the conclusions arrived at by the foregoing experiments a field at Cottenham, Cambridgeshire, broken up in the autumn of 1925, was thoroughly cultivated to free the land of weeds, and worked down to a fine tilth. On April 13th it was drilled with wheat in rows 3 ft. apart and 2–3 in. deep. On April 23rd examination of 3 one-yard samples yielded a total of 58 wireworms and when the experiment was concluded 8 representative one-yard samples yielded 119 wireworms or about 15 per yard, which by calculation indicated an assemblage of about 60,000 wireworms per acre. The interspaces relative to the 8 samples taken yielded 24 wireworms, or about 14,500 wireworms not assembled. The attraction figure would therefore be about 80 per cent. This experiment indicated that under ordinary field conditions considerable numbers of wireworms can be assembled at a suitable bait.

Experiments were carried out at Worthing and Cheshunt to test the possibilities of baiting land soon after breaking up, since for crops like strawberries successful baiting would enable the plants to be set out a year earlier. The trials were made in March–April within a month after breaking up the land. The undecayed sods made the setting of the baits a difficult process; in the previous experiments it had been possible to use a drill for many of the baits, but in these experiments it was necessary to set the baits by hand. At Worthing a variety of baits were

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planted in rows 20 yd. long and 3 ft. apart; and though the numbers of wireworms at the baits were low even after an exposure of 21 days, they give an indication of the relative efficiency of the different baits. Fourteen yard samples taken periodically between March 23rd and April 14th from each bait row yielded wireworms as follows:

Bait				No. of wireworms assembled
Wheat	...	...	...	45
Oats	...	...	...	24
Brewers' grains	...	...	...	15
Rape meal	...	...	...	10
Castor meal	...	...	...	7
Rape cake dust	...	...	...	9
Bran (wet)	...	...	...	8
„ (dry)	...	...	...	4
„ sweetened with sugar	...	...	...	7
„ sweetened with treacle	...	...	...	9
„ and wheat	...	...	...	11
Malt culms	...	...	...	4

It was interesting to note in this experiment that rape meal and rape dust, the bait usually recommended for attracting wireworms, proved very disappointing when compared with wheat and oats. Sweetening the bran did not increase its attractiveness to any extent, nor did mixing bran and wheat. This latter mixture has since been found very effective for short period baiting, but for long period baiting the mould development on the bran extends to the wheat and the bait no longer attracts the wireworms.

In the Cheshunt experiment wheat only was used as bait. It was planted on April 8th in rows 2 ft. apart and sampling on May 5th yielded 18 wireworms from 4 one-yard samples and on May 25th 7 samples yielded 39 wireworms or an average of about 5 per yard which, judging by the number of wireworms still present in the turf and the interspaces, was only a very small proportion of those present in the land.

The results from these two experiments showed that baiting could not be effectively carried out on new land until the greater part of the turf was broken down, a process requiring from 7 to 12 months, according to the character of the soil.

### (3) *Experiments in Glasshouses 1925-6.*

In the winter of 1925, when the downward migration of the wireworms prevented further work on baiting being conducted in the open, it was decided to carry out trials in glasshouses in Hertfordshire where wireworms had caused serious losses to tomatoes during the previous growing

season. The land had been broken up for glasshouse culture in the winter of 1924 and had carried its first crop of tomatoes in 1925 but there was still a considerable amount of undecayed turf which somewhat hampered the work. The soil in house No. 1 was sampled during the first week in December and 37 wireworms occurred in 40 samples 6 in. in diameter and 18 in. deep. In many cases the samples were taken to a depth of 21 in. but no wireworms were found at a depth greater than 12 in., the majority occurring within the top 9 in. of soil. The house was baited on December 9th and 10th with wheat, castor meal, brewers' grains, rape meal, bran and rape cake dust. A different system of applying the baits was adopted in order to test the relative efficiency of the bait substances under other conditions. The bait was set in holes made with a bulb planter, 2-3 in. deep and 2 ft. apart each way. A plot containing 30 sets was allotted for each bait and the whole series was taken in duplicate. At the time of setting the baits the soil temperature was 36° F. and the air temperature 32° F. Periodic examinations of the bait were made, but it was not until January 4th, 1926, that the wireworms began to assemble. On that date, when the soil temperature had risen to 47° F. one sample in each plot was examined and wireworms were found at the wheat, bran and castor meal. A week later more wireworms occurred in the samples taken and after that the assemblage was rapid and continuous. The final examinations were made on February 9th and the following table gives particulars of the relative efficiency of the baits used and the total number of wireworms from each plot.

Bait	No. of sets	No. of wireworms assembled
Wheat	60	429
Brewers' grains	60	85
Castor meal	60	73
Rape meal	59	71
Bran	59	68
Rape cake dust	60	63

As in the field experiments, wheat, the only "live" bait, proved outstandingly attractive while the remaining baits were comparatively inefficient. The periodic examinations of the baits showed that the wheat steadily increased in attractiveness throughout the time of exposure. In this experiment the wireworms collected were recorded in three groups; of the total of 789, 42 per cent. were over 18 mm. in length, 36 per cent. were 10-18 mm. long and 22 per cent. were under 10 mm.

The soil of No. 2 glasshouse, which was rather cloddy and contained

much undecayed turf, was sampled and yielded 57 wireworms from 64 samples 6 in. in diameter. On February 3rd it was baited with wheat and brewers' grains, the two baits which had proved most attractive to wireworms in the preceding experiment. The baits were set alternately in rows, rows 1-5 inclusive being placed 2 ft. apart and rows 6-10, 3 ft. apart. At mid-day on the date of sowing the soil temperature was 48° F. Gentle heat was turned on in the house after baiting and when the baits were sampled after 20 days' exposure the following figures were obtained:

Bait	Distance between rows ft.	No. of 1-yard samples	Wireworms assembled	Approx. No. of wireworms per yard
Wheat	2	19	262	14
"	3	12	193	16
Brewers' grains	2	12	45	4
"	3	15	61	4

Totalling the figures for each bait, 31 yd. of wheat row yielded 455 wireworms or an average of about 15 per yd. and 27 yd. of brewers' grains row yielded 106 wireworms or about 4 per yd.

In a glasshouse near Waltham Cross a further experiment was conducted on lines similar to the above and it was found that wheat attracted approximately six times as many wireworms as bran, rape or castor meal.

These experiments showed that in glasshouses under suitable conditions wireworms will assemble in considerable numbers at baits; and that, as in the field, germinating seed is by far the most attractive bait yet tried. As was noted in the open, soil conditions have considerable influence on the efficiency of the baits, for baits set in soil having a temperature of 32° F. failed to attract the wireworms until the soil temperature rose above 40° F.

#### (4) *Baiting wireworms among growing crops.*

Since wireworms exhibited marked preferences for certain of the baits used in the foregoing experiments, and since examination of weeds in wireworm infested land in Lincolnshire showed that the pest occurred commonly at the roots of some species and only rarely at the roots of others in close proximity, it was thought if an attractive bait were set between rows of crop plants known to be suffering from attack by wireworms, it might be possible to attract the insects and thus save the plants. With the assistance of Mr Kent, Horticultural Superintendent, Isle of Ely, permission was obtained to test this idea in a field of strawberries in the Wisbech area, known to be infested with wireworms. The

land had been ploughed up in the autumn of 1924 and the strawberries which were planted out in the autumn of 1925 had not made good growth, many having been killed during the winter and others obviously suffering from wireworm attack. From the field 19 soil samples 6 in. square and 8 in. deep yielded 64 wireworms or by calculation about 586,000 per acre. On April 9th, 1926, wheat, oats, bran and malt culms were set as bait between the rows of strawberries. Periodic examinations showed that the wireworms were assembling in large numbers. All the baits proved highly attractive for the first fortnight; wheat and oats, especially the latter, were increasingly attractive during the third week but bran and malt culms showed a decrease in efficiency during that period. The following table shows the number of wireworms assembled at the various baits:

Date 1926 April	Bait	No. of 1-yard samples	Wireworms present	Average per yard
16	Wheat	4	30	7.5
22	"	4	47	11.75
30	"	4	48	12.0
16	Oats	4	35	8.75
23	"	4	62	15.5
26	"	4	114	28.5
16	Malt culms	4	41	10.25
23	" "	4	35	8.75
30	" "	4	20	5.0
16	Bran	4	41	10.25
23	"	4	41	10.25
30	"	4	33	8.25

In order to determine to what extent the wireworms were leaving the strawberry plants for the baits, 5 sections, each 1 yd. square and containing 4 strawberry plants, their relative interspaces and the bait row, were examined 3 weeks after the setting of the bait and the following results obtained:

Plants	Section No. ...	Wireworms assembled					Total
		1	2	3	4	5	
4 strawberry plants and interspace		1	1	3	3	0	8
1 yard wheat bait		7	5	8	2	7	29
4 strawberry plants and interspace		3	5	3	0	1	12
1 yard wheat bait		11	9	31	6	3	60

Thus in an area containing 109 wireworms 89 or 81.6 per cent. were attracted from the plants on which they were feeding to bait set in rows between the crop.

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In May 1926 an experiment in baiting among crops was conducted on a flower farm in Cambridgeshire. On May 17th baits were drilled between rows of various kinds of flowers and examination on June 2nd gave the following figures:

Variety of plants	Bait	No. of 1-yard samples	Wireworms		Total
			Assembled	Interspace and at plant roots	
Pyrethrums interplanted with <i>Gypsophila</i>	Wheat	6	16	23	39
Pyrethrums	„	3	2	1	3
„	Rye	3	4	4	8
„	Oats	3	3	—	3
„	Bran	3	5	3	8
Gladioli	Wheat	3	12	3	15
Chrysanthemums	„	3	17	8	25
Asters	„	3	20	2	22
Statice	„	3	6	1	7
Calendula	„	3	3	5	8

These results show that out of a total of 138 wireworms present in the area, 88 or approximately 64 per cent. were attracted to the baits.

Vine borders in a glasshouse near Chislehurst were found to be heavily infested with wireworms which were seriously hampering the growth of the vines. Wheat and bran mixed 3 : 1 was set as bait on July 9th. The borders had previously been thoroughly watered and the soil temperature was 76° F. Examined 14 days later, 9 representative one-foot samples yielded 92 wireworms assembled and 4 wireworms in the relative interspaces, or an attraction of 96 per cent.

It is apparent from these experiments that baiting between growing crops has distinct possibilities as a means of attracting a considerable proportion of wireworms from the crop plants which are being attacked.

### (5) *Conditions for successful baiting.*

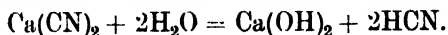
From the experiments herein described on baiting for wireworms in arable fields, in glasshouses, and between growing crops it is obvious that certain conditions are necessary for successful results. The baits should be set just prior to or during the height of wireworm activity near the surface of the soil; that is in autumn during October and November, or in spring during March, April and May. The land to be baited should be cleared of weeds which by affording other food for the wireworms might detract from the efficiency of the bait. The bait should be selected with due regard to the time available for its exposure; bran

and wheat are both quick acting baits but the former tends to lose its attractiveness after about a fortnight; wheat and oats both remain attractive for 3 weeks or more, the latter being less attractive during the first few days.

### III. BAITING AND CALCIUM CYANIDE FOR WIREWORM CONTROL.

From time to time various substances have been recommended as soil insecticides, and under ordinary farming conditions soot, salt, lime, kainit, various artificial manures, and naphthalene have been used and, though acting chiefly as repellants, they have occasionally been the means of temporarily relieving crops from wireworm attack. In 1922 calcium cyanide in various degrees of fineness became available in America and being a cheap and easily handled source of hydrocyanic acid gas, a potent insecticide, attracted the attention of economic entomologists, as a possible remedy for the control of soil insects; and Quale<sup>(15)</sup> published a brief note on this material as a soil fumigant in 1923. The first mention of calcium cyanide in connection with wireworm control was made by Horsfall<sup>(6)</sup> early in 1924 and since that time reports on its use against wireworms have been published by Campbell<sup>(2)</sup>, Spuler<sup>(20)</sup> and Horsfall and Thomas<sup>(7)</sup>. These investigators found that calcium cyanide was highly toxic to wireworms, and though rather expensive for field use, if it was used in conjunction with baiting was likely to prove a reasonable and efficient means of controlling this pest. Experiments conducted in various parts of England in 1925 and 1926 by the writers bear out the findings of the American workers.

Calcium cyanide,  $\text{Ca}(\text{CN})_2$ , is made from calcium cyanamid,  $\text{CaCN}_2$ , which is produced by a process of fixation of atmospheric nitrogen. It is quite distinct from cyanamid, having an extra carbon atom and totally different characteristics. When calcium cyanide is acted upon by atmospheric water vapour or soil moisture, hydrocyanic acid gas is given off; the reaction is expressed thus:



When this takes place the residue is lime and certain nitrogenous products which are beneficial to vegetation. In granular form crude calcium cyanide is bluish grey in colour, and about 90 per cent. passes through a sieve of 20 meshes to the inch and 40 per cent. through a 30 mesh sieve. In 10 samples of the commercial form used in these experiments analysis showed a minimum of 46.20 per cent. pure calcium cyanide, a maximum of 53.12 per cent., and an average of 49.1 per cent.



(1) *Preliminary tests.*

In order to determine the range of influence of calcium cyanide, experiments were conducted with wireworms in copper gauze cages. These cages were filled with soil and 5 wireworms put in each. The cages were then placed in the soil in various positions relative to a row of calcium cyanide granules drilled at the rate of  $3/8$  oz. per yd., the whole experiment being carried out in duplicate. Five days after the experiment was set up, the following results were obtained:

Position of cage	Wireworms					
	Dead		Alive		Moribund	
	A	B	A	B	A	B
Upon cyanide	5	5	0	0	0	0
Beside cyanide	5	5	0	0	0	0
1 in. from cyanide	5	5	0	0	0	0
2 in.    "    "	5	5	0	0	0	0
3 in.    "    "	2	2	2	3	1	0
Control cage	0	0	5	5	0	0

This experiment indicated that the lateral range for high toxicity was 2–3 in., and a similar experiment with cages 2 in. to the side and from 1 to 3 in. above, and 1 to 3 in. below the cyanide indicated efficient killing within this range.

(2) *Field Experiments in Lincolnshire 1925.*

In this series four experiments were conducted. The wireworm infested land was first cleared of weeds and then lightly cultivated and baited. After varying periods of exposure, during which examinations for assembling wireworms were made, calcium cyanide was applied to the bait rows by means of a hand-drill (Pl. XXV, fig. 1) to which a special deep plough attachment (*A*) had been fitted, thus enabling the cyanide to be deposited about 4 in. deep, *i.e.* just below the level of the baits. The drill had an indicator, by which the aperture adjustment was set according to the size of the seed to be sown. When the indicator was fixed at "Salsify" it was found to drill 60 yd. of bait row with 1 lb. of granular calcium cyanide. After the application of the cyanide, the land was lightly pressed to cover the cyanide thoroughly and to close the larger soil interspaces.

*Experiment No. 1* was designed to test the toxicity of calcium cyanide to wireworms under field conditions. On October 19th, 1925, after an

exposure of 14 days, 12 rows of bait were treated with calcium cyanide at the rate of 1 lb. per 60 yd. Rain had fallen the night before the application of the cyanide and the ground was therefore rather wet and somewhat adhesive, which interfered to some extent with even drilling. Four days after application the rows were sifted out and yielded a total of 299 wireworms, of which 143, or 47·8 per cent., were dead. From untreated rows from the check plot a total of 309 live wireworms were obtained.

*Experiment No. 2* was laid down to determine the influence of depth of baiting on the killing efficiency of calcium cyanide drilled at a depth of  $3\frac{1}{2}$  to 4 in. The baits were set on October 7th, left undisturbed for 14 days and cyanide drilled at the same rate as in Exp. 1. Six days after treatment the rows were sifted out and the results, which indicated that 3 in. is the most satisfactory depth to set bait, were as follows:

Depth of bait in.	Wireworms			% Killed
	Alive	Dead	Total	
2	51	28	79	35
3	35	30	65	46
4	52	25	77	32

In similar but untreated bait rows, 150 wireworms, including 1 dead specimen, were obtained.

*Experiment No. 3.* In this test six bait rows after being undisturbed for 14 days, were drilled with calcium cyanide as above, to determine the influence of time and exposure on toxicity. The first three rows were examined 6 days after treatment and the second three 12 days after treatment. The results showed 77·7 per cent. killed after 6 days and 78·5 per cent. after 12 days, indicating that under field conditions, calcium cyanide is likely to kill the majority of the insects within 6 days after application.

*Experiment No. 4.* Fifty rows of wheat 10 yd. long and 4 ft. apart were drilled on October 26th and 27th; on November 9th and 10th, calcium cyanide at 20 different rates between 34 lb. per acre (approximately 1 lb. per 100 yd.) and 143 lb. per acre was applied. The series was put down in duplicate and 10 rows receiving no calcium cyanide were left as a check plot. Examination 7 days after treatment yielded the results given in the accompanying table. From this it will be seen that a dressing of 73 lb. per acre, or 1 lb. per 50 yd. of bait row, gave over 90 per cent. kill of assembled wireworms and dressings over this amount gave, with one exception, kills of at least 95 per cent.

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Weight of calcium cyanide in lb. per acre	Wireworms			
	Alive	Dead	Total	% Killed
34	8	15	23	68
39	6	21	27	77
45	7	34	41	82
51	10	27	37	71
56	16	38	54	71
62	10	53	63	83
68	10	54	64	82
73	5	52	57	91
79	3	48	51	96
85	4	37	41	85
90	2	61	63	96
96	0	52	52	100
102	2	35	37	95
107	1	36	37	97
113	2	29	31	95
119	1	41	42	98
124	0	67	67	100
130	2	59	61	97
136	1	60	61	98
143	0	42	42	100
Nil	137	0	137	0

## (3) *Experiments in Glasshouses, 1926.*

At Hatfield a glasshouse, known to be heavily infested with wireworms, was baited on February 3rd. and the house gently heated. The soil was cloddy and when calcium cyanide at rates varying from 2-3½ lb. per 175 yd. was applied 14 days after baiting, these clods hampered the smooth running of the drill, hence the distribution of the cyanide was not quite even. Six days after treatment siftings of representative yard samples yielded results as follows:

Bait yards examined	Calcium cyanide per row of 175 yards lb.	Wireworms			
		Alive	Dead	Comatose	% Killed
10	2	25	40	14	50
12	2½	36	87	9	66
12	3	72	52	5	40
12	3½	28	70	8	66

A wireworm-infested glasshouse at Worthing was baited with wheat sown 2-3 in. deep in rows 2 ft. apart on February 24th, and gentle heat turned on in the house. Fourteen days later calcium cyanide at varying rates was applied, the rows of bait, readily located by the young shoots

of the wheat, being carefully followed with the drill. Six and seven days after the application of the cyanide, siftings yielded the results given below:

Amount of calcium cyanide per 150 ft. of bait row lb.	Wireworms		
	Alive	Dead	% Killed
$\frac{3}{4}$	65	117	64.28
1	51	195	79.26
1 $\frac{1}{4}$	22	111	83.45
1 $\frac{1}{2}$	9	144	94.11
1 $\frac{3}{4}$	12	149	92.54
2	19	141	86.50
2 $\frac{1}{4}$	0	151	100.00
Nil	232	0	0

(4) *Experiments amongst growing crops, 1926.*

Two experiments were conducted amongst growing crops to determine whether calcium cyanide could be used to control wireworms without causing injury to the crop.

A strawberry plantation at Wisbech, baited on April 8th, was dressed with granular calcium cyanide on May 4th at the rate of 2 $\frac{1}{2}$  lb. per 50 yd. of bait row. Examination of 10 one-yard samples 3 days after treatment yielded 59 wireworms. 1 was alive, 2 were moribund, and 56, or 95 per cent., were dead.

Vine borders under glass at Chislehurst were treated, after baiting, with calcium cyanide at the rate of 3 lb. per 50 yd. of bait row. Examination 3 days later gave a total of 97 wireworms including 92, or 95 per cent., dead specimens, from 11 separate one-foot samples.

These experiments are of special interest because they show that wireworms amongst growing crops can be controlled with calcium cyanide, for in neither case were the plants injured by the treatment.

(5) *Cost of treatment.*

The cost of treating land for the control of wireworms by the baiting and calcium cyanide method will depend on the value of the land and the type of crops to be grown. The spacing of the bait rows will be governed by the cropping, *e.g.* in glasshouses where a wireworm may cause the death of one or more tomato plants each capable of bearing 6-8 lb. of fruit, the bait rows should be close together so as to attract as many wireworms as possible. In such cases the cost of treatment will be higher because of the extra bait required, the longer time to

set the bait rows and the greater amount of calcium cyanide required. In the case of a block of tomato houses each 150 ft. long by 26 ft. wide, accommodating 12 rows of bait 2 ft. apart, each house would require  $1\frac{1}{2}$  st. of wheat costing about 2s. 6d. and with a hand-drill this would take about an hour and a half to set. The cost of baiting each house, therefore, would be about 4s. Calcium cyanide at the rate of  $1\frac{1}{2}$  lb. per 150 ft., or 18 lb. for the house, would be required, and cost about 1s. 2d. per lb., or 21s. for cyanide with a cost of application of 1s. 6d., making a total cost of 22s. 6d. for each house. This figure is interesting when compared with the methods usually adopted in these houses, which is to trap the wireworms with broccoli stems set in the ground to a depth of about 6 or 8 in., the woody base of the stalk and the roots protruding and serving as a handle for use when examining the traps. The broccoli stems are set between the rows of tomato plants usually about 2 ft. apart either way and are examined periodically, the wireworms boring into the stems being removed and destroyed. The cost of treatment in this manner worked out as follows for each house, the men being paid at the rate of 1s. per hour:

Preparing and setting bait, 1 man 2 hours ... .. 2s.

Examining baits, 4 men 3 hours, twice weekly for 4 weeks... 96s.

This treatment for each house, therefore, cost 98s. or about four times the cost of the baiting and calcium cyanide treatment. In some cases carrots are used as traps and under such circumstances, there would be the further cost of these to be added.

On agricultural land with wheat as bait drilled in rows about 3 ft. apart,  $3\frac{1}{2}$  st. is sufficient for an acre and about 90 lb. of calcium cyanide would be required, costing about £4. 10s. This, as an initial cost seems high but it must be remembered that the treatment will destroy wireworms which would otherwise be active and destructive for at least 3 years. The cost per year therefore over this period is not great, and in any case where intensive agriculture is followed, treatment at this expense might be adopted with advantage, though for general farming the cost might be considered prohibitive. An advantage of the baiting system is that by examining the bait it is possible to locate the main areas of wireworms infestation and only the infested portions of the field need be treated with the calcium cyanide.

## IV. CONCLUSIONS AND RECOMMENDATIONS.

Experimental work on the control of wireworms in various parts of England and in connection with both agricultural and horticultural crops indicate that a successful means of control consists of baiting infested land with wheat and oats, or a mixture of wheat and bran, and when the wireworms have assembled in the bait rows, applying calcium cyanide to the rows by means of a suitable drill. This method can also be used among growing crops when the space between the rows allows room for baiting. During the course of the work much experience has been gained as to the most suitable method of carrying out the process, and based on this the following tentative recommendations are made. The land should be cleared of weeds and the soil worked to as fine a tilth as possible a week or so before the bait is set. The bait, preferably wheat or oats, should be sown 2-3 in. deep in rows 2-4 ft. apart according to the value of the crop to be grown and the extent of wireworm infestation. The bait rows should be examined at about four-day intervals to note the rate of assembling of the wireworms, because the time of application of the calcium cyanide will depend on this. Under ordinary conditions maximum assemblage will take place in about a fortnight but should cold weather intervene a week or ten days longer may be necessary. The calcium cyanide can be satisfactorily applied with a hand-drill of the "Planet" Junior or "Norrahammar" type, fitted with a deep plough attachment, depositing the cyanide at a depth of about 4 in., *i.e.* below the level of the bait. About 2 lb. of calcium cyanide per 100 yd. of bait row is usually sufficient to ensure over 75 per cent. kill of assembled wireworms under ordinary field conditions. Under glasshouse conditions, where 100 per cent. kill is most desirable, 3 lb. per 100 yd. should be used. The calcium cyanide should be covered with soil immediately after application and the land rolled or pressed to close the larger air spaces and prevent the rapid escape of the hydrocyanic acid gas. Six or seven days after treatment the land can be lightly cultivated and opened up to liberate any residual fumes and plants can be set or seed sown within the next two days.

Spring seems to be the most satisfactory time of the year to adopt measures for the control of wireworms for the soil temperature is steadily rising and the wireworms becoming more active after the winter period and working back into the surface soil for feeding purposes. In the autumn, though numbers will assemble at baits, the steady fall of the soil temperature gradually induces a cessation of feeding and causes

the majority of the wireworms to migrate deeper into the soil. There is also risk of a sudden period of cold weather occurring during the exposure of the bait and driving the wireworms down immediately, so that the labour of baiting is wasted. If treatment must be carried out in autumn, it is best carried out in late September or early October. Calcium cyanide should not be applied when the land is wet, for drilling is difficult, even distribution almost impossible, and the drill constantly clogs, and, moreover, the hydrocyanic acid gas does not permeate the soil effectively. Land can be baited with success 7 or 8 months after breaking up from grass or ley; before this time has elapsed, the presence of turf annuls the influence of the baits. If such land must be treated for wireworms, calcium cyanide can be applied by means of a hopper fixed to the beam of an ordinary plough and regulated to sow the insecticide just in front of the falling furrow slice. Pl. XXV, fig. 2 illustrates the type of apparatus.

#### SUMMARY.

A study of the wireworm problem in Great Britain shows that this pest is especially important in areas devoted to intensive agriculture and commercial horticulture.

In addition to most farm crops, strawberries, various flowers and tomatoes are particularly susceptible to wireworm attack and because these crops so often occur on new land, losses due to the activities of the pest are frequent and serious. The nature and extent of wireworm attack, both in the field and in glasshouses, is described herein.

Examination of weeds growing in infested fields indicate that wireworms are found in numbers at the root of some plants such as couch-grass, *Agropyrum repens*, and yarrow, *Achillea millefolium*, while very few occur at the roots of plants like chickweed, *Stellaria media*, goutweed, *Chenopodium album* and annual nettle, *Urtica urens*.

Field observations on the movements of wireworms in the soil throughout the autumn, winter and spring indicate that there is a definite downward migration in autumn and an upward migration in spring. Correspondingly wireworm activity in the surface soil was noted to be at its height in September and October, and March, April and May.

Experiments on the use of baits as a means of assembling wireworms, indicated that large numbers will collect to baits such as wheat, oats, and bran, and moreover these baits can be used to attract wireworms from the roots of crop plants, on which they are feeding. Because of



Fig. 2. Tomato stem showing damage by wireworms ;  
three *in situ* (natural size).



Fig. 1. Potato tuber showing typical wireworm injury ( $\times \frac{1}{2}$ ).





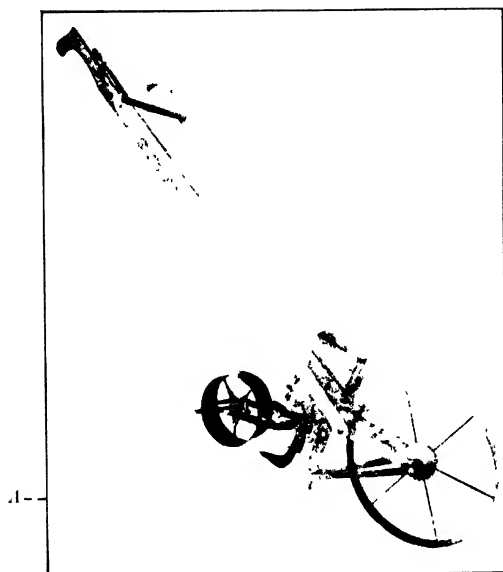


Fig. 1. 'Planet Jr.' hand-draw with deep plough attachment (1) as used for application of calcium cyanide



Fig. 2. Plough with plough-applicator attached as used for application of calcium cyanide at the time of ploughing.



the seasonal movements of wireworms, baiting is best carried out in autumn or spring.

Calcium cyanide in granular form has been found to be highly toxic to wireworms and used, in connection with the baiting, at the rate of 2-3 lb. per 100 yd. of bait row, destroyed 75-100 per cent. of the wireworms assembled.

Based on the results of experiments conducted in Lincolnshire, Hertfordshire, and West Sussex, during the years 1925 and 1926, and involving 10,000 wireworms, recommendations are made for the control of wireworms by means of baits and the use of calcium cyanide.

#### BIBLIOGRAPHY.

- (1) Board of Agriculture (1920). *Misc. Publ.* No. 23, p. 9.
- (2) CAMPBELL, R. A. (1924). *Journ. Econ. Ent.* XVIII, 562.
- (3) CURTIS, J. (1860). *Farm Insects*. London.
- (4) FORD, G. H. (1917). *Ann. App. Biol.* III, 97.
- (5) HAMMOND, J. (1912). *Journ. Agric. Sci.* IV, 386.
- (6) HORSFALL, J. L. (1924). *Journ. Econ. Ent.* XVII, 160.
- (7) HORSFALL, J. L. and THOMAS, C. A. (1926). *Journ. Econ. Ent.* XIX, 181.
- (8) LEES, A. H. (1916). *Ann. Rept. Long Ashton Res. Stn.* p. 37.
- (9) MIALI, H. W. (1902). *Injurious and Useful Insects*. London.
- (10) MILES, H. W. and PETHERBRIDGE, F. R. (1927). *Journ. Min. Agric.* XXXIII, No. 11, p. 931.
- (11) „ (1927). *Agricultural Progress*. IV, 11.
- (12) Ministry of Agriculture (1923). *Misc. Publ.* No. 39, p. 7.
- (13) MORRIS, H. M. (1922). *Ann. App. Biol.* IX, 2821.
- (14) ORMEROD, E. A. (1890). *Manual of Injurious Insects*. London.
- (15) QUALE, H. J. (1923). *Californ. Univer. Coll. of Agric. Rept.* 1922-3.
- (16) ROBERTS, A. W. R. (1919). *Ann. App. Biol.* VI, 116.
- (17) „ (1921). *Ibid.* VIII, 193.
- (18) „ (1922). *Ibid.* IX, 306.
- (19) ROEBUCK, A. (1924). *Journ. Min. Agric.* XXX, No. 2.
- (20) SPULER, A. (1925). *Journ. Econ. Ent.* XVIII, 702.
- (21) TATTERSFIELD, F. and ROBERTS, A. W. R. (1920). *Journ. Agric. Sci.* x, 199.
- (22) THEOBALD and MCGOWAN (1916). *Journ. Board of Agric.* Supplement, No. 15.
- (23) THOMPSON, M. (1924). *Ann. App. Biol.* XI, 349.
- (24) WARDLE, A. R. and BUCKLE, P. (1923). *Principles of Insect Control*. Manchester.

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## REVIEWS

*The Biological Relations of Optically Isomeric Substances.* By ARTHUR R. CUSHNY. Baillière, Tindall and Cox. Pp. vi + 80. \$2.00.

This small book, the product of the Dohme Memorial Lectureship, and the last published work of the late Prof. Cushny, makes profoundly stimulating reading. The peculiar value of such lectureships as these is, that they constitute a platform from which acknowledged masters in various fields of scientific endeavour can present a fairly complete view of some of the problems met with in the course of their work, and enable a partial synthesis to be made of results of researches scattered through many journals. Their influence in many cases spreads into new fields. It appears to have been the hope of Prof. Cushny that this should be the case, for whereas this borderland subject has proved of a certain interest to chemists and of importance to the pharmacologist, the biologist, in general, has held almost entirely aloof from the consideration of the bearing optical activity may have on the phenomena exhibited by living matter. This, perhaps, is the more remarkable in view of the fact that Pasteur, from a starting point dealing with the crystallographic relationships of the optical isomers of tartaric acid, found them a direct pathway to his great work in biology and medicine.

The admirable historical introduction may prove to the purely biological reader as fruitful a portion of the book as any. The selective synthesis of optically active compounds and their origin in nature, which have an interest in the evolution of living matter, is dealt with succinctly and suggestively. This naturally leads to the selective action of enzymes and the preferential growth of micro-organisms on one of the pair of isomers—the well-known lock and key effect of the biochemist, and to the decomposition of the isomers in living tissues. To the general physiologist these facts constitute a body of important knowledge, but a definite query arises as to whether the mycologist and bacteriologist have exploited this field of work as critically and thoroughly as it unquestionably deserves, for here are processes susceptible of exact quantitative measurement which, at first sight, would seem to be susceptible to the differentiation of strains.

Of necessity the subject-matter is mainly devoted to an account of the relationship existing between optical activity and configuration on pharmacological and toxic action. But here again the author takes as wide a view of the matter as possible, and some attempt is made to draw parallelisms between this phase of the subject and other biological effects indicated above and certain well-known biochemical methods of preferential precipitation. Analogies of this order are not dealt with in any uncritical way and throughout the book individual researches and opinions are evaluated with considerable spirit.

These lectures also contain an entirely welcome reminder of the difficulties of applying some one chemical or physical concept to pharmacological and physiological action, and that the physiological activity of a chemical substance is seldom purely chemical or purely physical.

The lectures end on the note that the methods required for further advance are rather those of chemistry than biology. The reviewer feels, that however true this may be from a purely pharmacological aspect, a separation into watertight compartments of workers in this field would be little short of disastrous.

Prof. Cushny's expressed aim in choosing the subject of these lectures was to attract to it more attention and lead others to advance it further. On closing the book one regrets deeply that his untimely death has removed a pioneer guide and powerful influence to this end.

F. TATTERSFIELD.

*Enzymes: Properties, Distribution, Methods and Applications.* By SELMAN A. WAKSMAN and WILBURT C. DAVISON. London: Baillière, Tindall and Cox, 1926. Pp. xii + 364. 25s. net.

This volume does not pretend to be an original contribution to the subject but is the outcome of an endeavour to collect in as concise a form as possible the available information in regard to enzymes and to indicate the sources from which more detailed knowledge may be obtained. With this aim the authors have consulted over 2000 papers on the subject and pieced together the irregular and loosely fitting fragments into some kind of a coherent mosaic. Particular attention has been paid to the occurrence and preparation of enzymes, to the methods of measurement, and to the practical applications of enzymatic activity—phases of the subject that are frequently overlooked in volumes on plant and animal physiology and even in more specific treatises.

The book is divided into four portions. The first, dealing in general with the properties of enzymes, contains an historical introductory chapter followed by chapters on enzymes in biological processes; the chemistry of enzymes and enzymatic reactions; factors which influence enzymatic reactions. The second portion deals in general with the distribution of enzymes and contains chapters on enzymes of the human and animal body; plant enzymes; enzymes of micro-organisms. The third portion discusses methods for the preparation and study of enzymes, containing chapters on the preparation, preservation, purification and measurement of enzymes; enzymes acting upon fats and esters (esterases); enzymes acting upon carbohydrates and their derivatives; enzymes acting upon proteins and their derivatives; oxidases and oxido-reductases; zymases; catalase. The fourth portion consists of a single chapter on the practical applications of enzyme activity. There follow 78 pages of references and an Index. The whole value of a book of this nature is in the ready availability of its data and this to a very large extent depends upon its Index. In the present volume the Index is not nearly detailed enough and does not give a fair idea of the mass of data the volume contains: its incompleteness seriously impairs the practical use of the book for reference. The volume is one for personal possession and the insertion of one's own annotations and a drawback to this is the price. For a lightly bound book of 364 pages of almost straightforward type-setting plus a few graphs, 25s. is too high a price and will prevent many who would find it very useful in their work from purchasing the volume.

The amount of literature in these borderline regions is becoming so vast that unless workers are, from time to time willing to sacrifice time and energy in collating scattered work and making it available in such form as this, scientific research will be crushed by its own weight and incoherence. In the present case an associate professor of soil microbiology in an Agricultural Experiment Station and an associate professor of pediatrics in a famous Medical School have combined to this end. Such a collaboration is indeed a promising sign of the times, and it is to be hoped that it foreshadows a bridging of the gulf between medical men and workers in the cognate branches of agricultural science. Both have much to gain and nothing to lose by a pooling of their knowledge and ways.

WILLIAM B. BRIERLEY.

*The Composition and Distribution of the Protozoan Fauna of the Soil.* By H. SANDON. Edinburgh and London: Oliver and Boyd, Biological Monographs and Manuals, 1927. Pp. xv + 237 with 6 Plates, 11 Tables, 2 Text-figures and 3 Charts. 15s. net.

A new chapter of interest in soil microbiology and in the protozoa was opened in 1909 when Russell and Hutchinson advanced their hypothesis on the part played by the protozoa in the life of the soil. They showed that partial sterilisation of soil by heat or volatile antiseptics was followed by large increases in the numbers of

ammonifying bacteria. In seeking for an explanation of this rise in bacterial numbers they discovered that the protozoa normally present in an untreated soil had completely or almost completely disappeared from the partially sterilised soil. A limiting factor had been removed and since some of the protozoa were known to ingest and feed upon bacteria when living in cultures, the suggestion was made that such protozoa might function in the soil as the factor limiting bacterial numbers.

Zoologists had known for many years that several kinds of protozoa could be found in fresh water and on moist vegetation, mosses for example, close to the soil surface but it was not known that they were to be found normally inhabiting arable and pasture soils. Moreover it was a distinctly novel and striking suggestion that they were probably functioning in the soil as consumers of bacteria and therefore limiting its fertility.

Many questions concerning them and their life in the soil called for answers and most promising lines of biological investigations were opened up. We need not go into details of all these but consider some that are relevant to the present purpose. Were the protozoa world-wide in their distribution and how far down in the soil could they be found? Did they occur in all kinds of soil and what kinds of Protozoa were to be found? Were they entirely new to science or did they belong to genera and species already known? These questions are discussed in the book under review which is the latest to be added to the admirable series of Biological Monographs and Manuals. The book is divided into two parts. The first 69 pages are devoted to the general biology of the soil protozoa considered under various aspects whilst the remainder is purely zoological and consists of brief descriptions of all the protozoa so far recorded from soil systematically classified.

In the opening section methods for the study of soil protozoa are first given. Then follow notes on the large collection of soils from various parts of the world which have been examined for their presence. The results of this examination are set out in tabular form along with figures for total nitrogen, moisture content and pH values. Under the head of "Influences affecting Protozoan Fauna of Soils" a number of very interesting points are discussed such as geographical influences, rainfall, influence of vegetation, irrigation, soil type, soil bacteria, vertical distribution of protozoa in the soil and physical and chemical properties of the soil. Correlations between the different groups of protozoa and between the numbers of protozoan species and nitrogen content, hygroscopic moisture and pH value are also examined. All this is simply written and in a well-balanced manner particularly the concluding discussion, pp. 55-58. The only criticism we have to offer of the first part of the book is that it is made somewhat difficult to read and carry in the mind because of the way in which it is set out. The reason being that the same weight of type is used throughout for cross-headings whether for principal or for subsidiary sections. It is probable that the matter could have been more suitably presented to the reader by throwing it into chapter form with appropriate sectional and sub-sectional headings.

Introducing the zoological part of the work there is an account of the soil protozoal community in which it is shown that the majority of the 250 species so far found in the soil have been recorded from other habitats also, whilst 21 species only belong peculiarly to the soil.

The descriptions of the species are brief, adequate and to the point and after each main group, Mastigophora, Rhizopoda and Ciliata there is a very full table, bewildering one had almost said, to facilitate the running down of any given species belonging to the group.

In bringing so much scattered information into a handy form the book will be indispensable to the investigator in soil microbiology and to the general biologist desirous of studying the subject. Its value would, we think, have been even greater had it been possible to find room for more drawings of protozoa. The six plates are good and the figures clear and not crowded whilst particularly useful is Plate 1 showing the appearance of the cysts of some of the commoner forms, but we should have liked to see more figures even had it meant sacrificing one or two tables or possibly the three charts at the end of the book. The time and labour expended on the latter might, we feel, have been devoted to giving us more drawings.

There is a good bibliography and the whole work is happily almost completely free from errors or misprints. We have, however, noted the following: p. 73, Plate IV should be Plate II and p. 186, Butochli is used in error for Butschli.

T. GOODEY.

*Hydrogen Ion Concentration. Its Significance in the Biological Sciences and Methods for its Determination. Volume 1. Principles of the Theory.* By LEONAR MICHAELIS. Authorised translation from the second revised and enlarged German edition, by W. A. PERLZWEIG. London: Baillière, Tindall and Cox. 1926. Pp. xiv + 299. 32 Figures. 22s. 6d.

Michaelis has been one of the pioneers in the application of physico-chemical methods to the study of biological systems. His *Wasserstoffionenkonzentration*, published in 1912, was the first and for many years the only comprehensive treatment of the most fruitful section of this work—the definition and measurement of the intensity factor of acidity and alkalinity. The subject developed so rapidly both on the theoretical and applied sides that the second edition was planned in three separate volumes dealing respectively with theoretical foundations, the methods of measurement and the applications, especially to colloid chemistry and physiology. Volume I on “The Principles of the Theory” was published in German in 1921, but the translation, here reviewed, includes a few brief discussions of some of the more significant advances in the intervening five years. Unfortunately Volumes II and III have not yet appeared. Although these applied sections may appeal more directly to the biologist, some acquaintance with the theoretical principles is clearly essential if measurements of the complex systems of biology are to be accurately performed and correctly interpreted. In the first half of the book the chemical equilibria of the ions are presented clearly and in detail. The second half on the ions, especially the hydrogen ions, as sources of electrical potential differences is more novel; an attempt is made to build up into a single coherent structure a series of facts and theories derived from isolated physical, chemical and physiological investigations of these phenomena. Unity of treatment has been maintained at the expense of the literature references.

In Chapter I the results of the application of the simple mass action law to the electrolytic dissociation of water and weak acids and bases are brought out with particular clearness by means of the extensive use of dissociation and dissociation-residue curves. These lead easily to the amphoteric electrolytes and the concept of the isoelectric point. The influence of the hydrogen-ion concentration on the solubility of slightly soluble compounds of special biological interest, *e.g.* calcium carbonate and uric acid, is treated in detail. It may be noted that the translator follows the convenient American convention of writing Sørensen's symbol as pH.

The significance of titratable acidity, pH value and buffer action in the measurement of acidity are briefly discussed. Chapter III forms a particularly useful introduction to the most difficult aspect of the ionic theory—the “anomaly of strong electrolytes.” Neutral salts do not obey the simple mass-action law, if their degree of dissociation is estimated, as in the case of weak electrolytes, by means of the electrical conductivities of their solutions. They are now regarded as completely dissociated but influenced by electrical interionic forces and hydration. Further they appear to increase the degree of dissociation of weak electrolytes, so that in physiological processes the absolute dissociation constants of the weak electrolytes will need to be replaced by constants reduced to the physiological salt contents. Michaelis's treatment is essentially historical, but it is sufficient to show where caution is necessary and to indicate the nature of the modifications that the newer theories may introduce.

The effects of a possible actual salt formation and electrolytic dissociation in non-aqueous solutions are briefly considered in Chapters IV and V.



Although all solutions of electrolytes contain equal amounts of positively and negatively charged ions, when considered in bulk, slight inequalities may occur at the boundaries of different phases. The large amounts of free electricity involved in such separations of the oppositely charged ions give rise to a number of electrical phenomena which may have important physiological bearings. The potential differences between metallic electrodes and solutions (Chapter IV) provides the basis for the electrometric estimation of hydrogen ion concentration by the hydrogen or the quinhydrone electrode. Diffusion potentials (Chapter VII) occur at the junction of two aqueous solutions of different composition, but they are of little physiological significance and may easily be eliminated by the potassium chloride bridge in electrometric  $pH$  measurements. Chapter VIII describes the potential differences that arise at the boundary of two phases in consequence of the limitation imposed by electrostatic effects on the distribution of the ions between the phases, as in Beutner's oil-water chains, and Chapter IX those between solutions separated by a membrane impermeable to one of the ions, the Donnan effect.

A lengthy final chapter attempts to bring order out of the confusing subject of adsorption. No fundamental contrast exists between the so-called chemical and physical forces involved in chemical union and adsorption. The exceptional case of adsorption of the hydrogen and hydroxyl ions leads to the classification of substances, not in state of molecular dispersion, as acidoid, basoid, and ampholytoid according as they show analogies with acids, bases and ampholytes. Electrokinetic processes such as the movement of suspended particles and the passage of water through a diaphragm under the influence of applied voltages are discussed in considerable detail in connection with the adsorption of ions. The distinction between these adsorption potentials and the true phase boundary potentials is emphasised.

This book will undoubtedly take its place as an indispensable reference book to be used in conjunction with a practical treatise such as Clark's *Determination of Hydrogen Ions*, by all workers, whether biologists or chemists, concerned with the better understanding of the chemical processes in living organisms and their environments.

E. M. CROWTHER.

## COMPARISON BETWEEN KAURI PINE AND SWAMP KAURI (*AGATHIS AUSTRALIS*)

By J. F. MARTLEY, A.R.C.S., M.Sc.

(*Forest Products Research Laboratory. Department of  
Scientific and Industrial Research.*)

OWING to the decreasing supplies of kauri pine the market has had recourse to the supplies of swamp kauri in order to keep pace with the demand for this valuable New Zealand timber.

Swamp kauri is the name given in the trade to the timber milled from the buried logs of kauri pines (*Agathis australis*) which flourished in the distant past and which have been preserved to the present day by being submerged in their native swamps. The high price of kauri pine has made the extraction and utilisation of these logs a remunerative business, especially as it is difficult to distinguish with any degree of certainty between swamp kauri and kauri derived from trees grown in the present time.

As users of kauri pine are doubtful whether the qualities of swamp kauri approach the excellence of those of kauri pine it has been necessary to examine a number of specimens in order to find if it is possible to distinguish the timber derived from felled logs from that obtained from the submerged logs.

The material for examination consisted of some ten reputed samples of various sizes of kauri pine, and of four samples of reputed swamp kauri which had been obtained by the Forest Products Research Laboratory through the courtesy of various London firms. All the samples had been air dried and several of the specimens had been in storage for a considerable number of years. Unfortunately no determinations were made of the moisture content of the samples when they were received, but probably none had a moisture content in excess of about 25 per cent.

Comparisons between swamp and normal kauri were confined to an examination for structural differences observable under the microscope, to the measurement of the amount of swelling and shrinking ("working") with change of moisture content, and to the determination of the moisture content when in equilibrium with an atmosphere of a given

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relative humidity and of the density of the wood substance composing the different samples.

In the microscopic examination no consistent difference could be found between the normal and the swamp kauri. In both the number of resin tracheids was substantially the same. The only difference that could be noted was that the number of tracheids with inclined striae on their walls was slightly greater in the swamp material than in the normal though in neither case were these markings either numerous or pronounced.

Table I.

*Kauri pine. Data on the shrinkage of five specimens derived from two samples of kauri pine on being oven dried at 105° C.*

	Kauri 1		Kauri 2		
	a	b	a	b	c
Weight in gm.	16.994	16.415	25.074	27.823	29.751
Volume in cm. <sup>3</sup>	29.31	28.39	40.23	43.50	48.48
Density	.580	.578	.623	.640	.614
% moisture content	14.09	14.13	10.89	11.22	11.07
Oven dry weight	14.896	14.383	22.616	25.016	27.787
Oven dry volume	27.29	26.39	38.68	42.21	46.90
Oven dry density	.546	.545	.585	.593	.571
Moisture loss in gm.	2.098	2.032	2.458	2.807	2.964
Volume loss in cm. <sup>3</sup>	2.02	2.00	1.55	1.39	1.58
Volume change per } loss of 1 gm. } moisture	.963	.984	.631	.495	.506

Table II.

*Swamp kauri. Data on the shrinkage of six specimens, three from each of two samples of swamp kauri, on being oven dried at 105° C.*

	Swamp kauri 1			Swamp kauri 2		
	a	b	c	a	b	c
Weight in gm.	31.465	32.650	31.490	54.980	53.208	53.392
Volume in cm. <sup>3</sup>	53.59	54.76	52.50	89.91	87.78	89.13
Density	.587	.596	.600	.615	.606	.598
% moisture content	13.82	13.71	13.35	13.05	12.86	12.65
Oven dry weight	27.645	28.712	27.782	48.634	47.144	47.396
Oven dry volume	51.57	52.94	50.78	86.20	85.94	86.40
Oven dry density	.536	.542	.547	.564	.549	.549
Moisture loss in gm.	3.820	3.938	3.708	6.346	6.064	5.996
Volume loss in cm. <sup>3</sup>	2.02	1.82	1.72	3.71	1.84	2.73
Volume change per } loss of 1 gm. } moisture	.529	.462	.464	.585	.303	.455

The comparisons of the changes in volume which occurred in the two types of material on change of moisture content were made by measuring the volume of a number of rectangular blocks of about 50 cm.<sup>3</sup> in size, in the air dry state under current laboratory conditions and again after drying in an oven at 105° C.

Two samples of normal kauri were taken and two of swamp kauri and measurements were made, except in one case, of the dimensions of three rectangular blocks prepared from each of the four samples. Each dimension of a block was taken as the mean of the measurements made at nine different points with a screw gauge measuring to 1/50 mm. The results of the measurements made before and after drying are given in Tables I and II, in the last line of which will be found the shrinkage in volume that the blocks suffered on losing one gramme of moisture.

It is seen from the figures that although there is a certain amount of overlapping among the figures, the shrinkage of swamp kauri is definitely less than that of normal wood losing moisture under the same conditions. Although the shrinkage was measured over a different range of moisture content, this result is in agreement with the finding of Janka<sup>(1)</sup> that wood which had been soaked for a period of one and a half to three years in salt or in fresh water shrank less on drying from the wet to the air dry condition than green wood which had not been so treated.

The hygroscopicity measurements were made by suspending a slip of wood, 0.50 mm. thick and weighing about 0.10 gm. from a calibrated spring supported in a glass vessel made by cementing together pieces of sheet glass of suitable size. The relative humidity in a vessel was kept at a constant value by the presence of some suitable salt moistened with water. The extension of the spring was measured with a travelling microscope reading to 1/50th mm., when the wood had reached the moisture content in equilibrium with the atmosphere in the vessel. From the oven dry weight of the slip of wood which was determined subsequently, and the spring calibration chart it was a simple matter to calculate the moisture content in the different relative humidities. The sensitivity of the springs used was such that with a load of about 0.10 gm. an extension of 1/50th mm. was equivalent to a difference in moisture content of somewhat less than 0.04 per cent.

The following salts were used to condition the air within the vessels: sodium chloride and the hexahydrate of calcium chloride in the presence of a small quantity of water, and fused calcium chloride. The relative humidities over these salts at laboratory temperatures are approximately 75, 35 and 3 per cent. respectively—Obermiller<sup>(2)</sup>.

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The spring extension was found for each of the two specimens of normal kauri pine and swamp kauri in the successive relative humidities of the following cycle: 75, 35, 3, 35 and 75 per cent., by transferring the specimen while still suspended from its spring to the next vessel after equilibrium had been reached in the previous atmosphere.

Table III.

*Comparison of the equilibrium moisture content of two specimens each of kauri pine and of swamp kauri over the given relative humidity cycle. Temperatures at the time of equilibrium given in each case.*

Salt	Approximate relative humidity %	Kauri pine				Swamp kauri			
		No. 2		No. 3		No. 2		No. 3	
		% M.C.	°C.	% M.C.	°C.	% M.C.	°C.	% M.C.	°C.
1. NaCl	75	15.0	13	15.7	12	16.3	16	15.9	16
2. CaCl <sub>2</sub> , 6H <sub>2</sub> O	35	11.4	11	11.9	12	12.1	16	11.0	17
3. CaCl <sub>2</sub>	3	6.4	12	6.6	15	4.3	17	2.6	17
4. CaCl <sub>2</sub> , 6H <sub>2</sub> O	35	9.9	13	10.1	18	9.8	15	9.4	17
5. NaCl	75	15.1	13	15.7	16	16.0	13	15.8	17

The moisture contents of the samples of normal and swamp kauri in the successive stages of the above relative humidity cycle are given in Table III. No attempt was made to keep the temperature constant, but in the table the temperature of the air close to the vessel is given at the time of measurement of the spring extension. On the analogy with the effect of temperature on the regain of cotton and other materials, Wilson and Fuwa<sup>(6)</sup>, the temperature effect in these experiments will be less than the experimental variation.

It will be seen from the figures in the table that there is no constant difference between the normal and the swamp kauri as far as regards hygroscopicity. The reason for the considerable differences in the moisture content of the samples in the atmosphere controlled by "dry" calcium chloride is that sufficient care was not taken to see that the calcium chloride was sufficiently dry in the earlier experiments.

The most interesting feature disclosed by the figures is the marked hysteresis shown on the return cycle over the hydrated calcium chloride (35 per cent. relative humidity) which, however, practically vanishes when the atmosphere of 75 per cent. relative humidity is again attained. This agrees with the latest work on the regain of cotton in which it is shown that absorption and desorption are reversible over short portions of the regain curves in the neighbourhood of saturation and of zero moisture content—Urquhart and Williams<sup>(3)</sup>.

The last property which was investigated was density of wood substance and here also no decided difference was discoverable between the two classes of material, although the swamp kauri had on the average a somewhat lower density.

Densities were determined as follows. About 5 gm. of the sample was cut up into small cubes of sides of about 1 to 2 mm. The material was then waterlogged by soaking under distilled water under reduced pressure for a week to ten days to remove the contained air. The material was then weighed in a specific gravity bottle of 50 cm.<sup>3</sup> capacity in boiled distilled water. After determining the oven dry weight the density of the wood substance of the sample of kauri was calculated at 15° C. after making any requisite temperature corrections.

Table IV.

*Results of the determinations of the density of wood substance at 15° C. of ten samples of kauri and four of swamp kauri. For comparison are given the determinations for three samples of common birch.*

Kauri pine	Swamp kauri	Common birch
1.485	1.494	1.512
1.513	1.504	1.502
1.512	1.497	1.520
1.525	1.516	—
1.534	—	—
1.486	—	—
1.532	—	—
1.530	—	—
1.495	—	—
1.503	—	—
1.512	1.503	1.511

The results of the determinations for ten samples of normal kauri and for four of swamp kauri are given in Table IV, which shows that on the average the density of the wood substance of swamp kauri is less than 1 per cent. lighter than that of the normal kauri. For comparison figures are given for three samples of the common birch (*Betula alba*).

The figure obtained for the density of wood substance by this method is not necessarily that of the wood substance itself but is the mean density of all the solid material of which the wood as a whole is composed. It must also be remembered that the density measured is that of wood substance in water which is about 5 per cent. higher than that of the dry material.

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Similarly to starch, gelatine and other colloids when wood substance absorbs moisture the final volume of the damp material is somewhat less than the sum of the separate volumes of the material and of the moisture absorbed owing to the volume contraction that occurs on wetting—Volbehr(4).

In some recent work on swamp kauri Welch(5) has shown that kauri milled from these buried logs is liable to become worthless, due to damage which develops during seasoning and which reduced the tensile strength to the same value as the compression strength. This reduction in strength he attributes to the spiral checks or cracks which appear on the tracheid walls during the process of seasoning (presumably artificial seasoning). Some of Janka's experiments(1) are of interest in this connection since he showed that a period of two to three years' soaking in fresh or in salt water definitely reduced the compression strength of timber by somewhat over 5 per cent. when tested subsequently in the air dry condition; approximately 13 per cent. moisture content. He made, however, no microscopical comparison between the soaked timber and the controls.

Welch found no difference in the ash content, or in the rate of moisture loss or of moisture absorption between kauri pine and the swamp kauri.

#### SUMMARY.

Swamp kauri was compared with normal kauri pine wood but no very decided differences between them were discoverable.

No definite structural differences were noted in the microscopic examination.

The shrinkage in swamp kauri was found to be less than in normal kauri when losing moisture under the same conditions.

No differences in hygroscopicity were noted.

The density of the wood substance of swamp kauri is the same or slightly less than that of normal kauri pine.

No mechanical tests were made.

The above investigation was carried out by the Forest Products Research Laboratory. I am indebted to the Director, Mr R. S. Pearson, C.I.E., F.L.S., for granting permission for publication.

## REFERENCES.

- (1) JANKA, G. (1907). Die Einwirkung von Suss- und Salzwässern auf die gewerblichen Eigenschaften der Hauptholzarten. *Mitt. a.d. forstl. Versuchswesen Österr. Wien*.
- (2) OBERMILLER, J. (1924). Die technisch durchführbare Einstellung—eines beliebigen Luftfeuchtigkeitsgrad. *Zts. ang. Chem.* xxxvii, 904 6.
- (3) URQUHART, A. R. and WILLIAMS, A. M. (1920). A review of work on the absorption and desorption of moisture by textile materials. *Trans. Faraday Soc.* xx, pt. 2.
- (4) VOLBEHR, B. (1896). *Untersuchungen über die Quellung der Holzfaser*. Inaug. Dissert. Kiel.
- (5) WELCH, M. B. (Dec. 1926). An examination of defective New Zealand kauri (*Agathis australis*). *Proc. Roy. Soc. N.S.W.*
- (6) WILSON and FUWA (1922). Humidity equilibria of various common substances. *Journ. Indust. Engin. Chem.* xiv, 913.

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## YIELD STUDIES IN OATS

### II. THE RELATIONSHIP BETWEEN THE CHARACTERISTICS OF A SEED SAMPLE—ITS GERMINATION AND FIELD ESTAB- LISHMENT—AND THE EARLY GROWTH OF THE SUBSEQUENT PLANTS

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#### I. INTRODUCTION.

In a previous paper (7) reporting upon yield studies in oats, the influence of the parent crop upon the seed produced, its germination and subsequent growth, was shown to be one of the factors influencing the growth and yield of this cereal. It was found, for example, that the altitude at which the parent crop was grown influenced the panicle organisation and the quality and quantity of the grain produced; time of sowing of the parent crop was also shown to have an effect upon the seed qualities. The present paper forms a continuation of that study; the influence of the seed characters of commercial samples are considered.

Under normal economic conditions it is rarely an easy matter for the practical grower who purchases seed to obtain reliable information of potential value, as to the growth and environment of the parent crop; it is not always even possible to trace the parent crop. The farmer is therefore faced with the problem of selecting seed without such knowledge and is often forced to rely upon his own judgment of the seed as gauged by its apparent qualities re-enforced by germination data which are obtained through the provisions laid down in the Seed Act.

A study of a number of seed samples—of one variety, Record, believed to be a genetically pure type—has been undertaken in order to evaluate the importance from the standpoint of producing a good yield of those seed characters, which can be more easily ascertained.

For this purpose a number of samples from different environments were collected—and Wales possessing widely differing crop environments is a very suitable area from which to select. These samples were analysed and then grown together under normal conditions, so that growth data could be obtained.

## II. THE COLLECTION OF THE SEED SAMPLES FROM DIFFERENT ENVIRONMENTS.

Twenty-five samples of grain were obtained from crops of Record oats grown in every Welsh county in the year 1926: some were from fertile lowland, others had been grown at surprisingly high elevations for *Avena sativa*. Although an attempt was made to obtain a natural sample as harvested, the absence of small grain (produced by the second flowers of the spikelets) in several cases indicated that some process of mass selection had been carried out in those samples particularly rich in large ("first") grain. For such reasons it was not possible to obtain all the data desired bearing on the question of the effect of "source of origin" upon the grain sample. Despite this, some interesting figures were observed in particular cases. From a crop grown at an elevation of 1200 feet above sea-level in the Llanarnion Duffryn Ceiriog district of Denbighshire the grain sample consisted almost entirely of extremely heavy first grain (no doubt some small second grain had been separated). The average weight per 100 dry caryopses of this sample was 31.69 gm. contrasting with a general figure of from 20–24 gm. It would appear that the number of grains per spikelet was reduced at this elevation. No data as to the number of spikelets per plant were obtained, but from previous work<sup>(6)</sup> it would seem probable that this number would be also reduced.

The moisture content of the samples varied from 12.5 to 17.2 per cent. The percentage husk varied from 23 to 28.5 per cent. Twenty-four of the samples were true to type—one was discarded on morphological grounds. A sample from a crop harvested in wet weather and stored so that the grain became heated was deliberately included in the series. This sample (Cc. 3069) gave very low germination figures and was outstanding on that account, so different was it from the remaining 23 samples (*i.e.* the deviation from the mean being exceptionally large) that frequently this sample determined the nature of the correlation coefficient between two sets of data. Calculations, omitting this sample (Cc. 3069), also have been made and wherever data for 23 samples are presented this is the one low germinating sample that has been omitted. By its very low germination and establishment figures a helpful extreme case has been provided for study.

### III. THE METHODS ADOPTED FOR THE ANALYSIS OF THE SEED SAMPLE; COLLECTION OF SEED DATA.

(a) *Weight per 1000 grains.* The representative samples were carefully drawn by the approved method of the seed-testing stations and the average figure employed.

(b) *Moisture content, percentage husk and dry weight of caryopsis.* After husk removal of a sample of approximately 5 gm. weight, the caryopses were rapidly ground up and dried in an oven at 95° C. until constant weights were obtained. Drying *in vacuo* could not be carried out with so many samples (approximately 200). It was found that higher temperatures caused very slight increase in weight in the case of the ground caryopses if the drying was too prolonged, due no doubt to some oxidation. All moisture estimations both of the husk and caryopses were carried out in triplicate series. The percentage of husk to total is calculated upon the dry weight basis although it was only rarely found that the husk and caryopses differed in moisture content.

(c) *Sand germination tests—heating the grain.* These tests were carried out by the standard seed-testing method, using glazed earthenware saucers covered by slate. Five replications, *i.e.* five separate hundreds, of each sample were tested together. Another series of 500 seeds, which had been previously heated for 15 minutes at a temperature of 75° C. with all the grain spread out so as to acquire rapidly the oven temperature, was also tested.

## IV. THE COLLECTION OF THE GROWTH DATA.

Five replicated rows of each sample of grain, both heated and control, were grown together in a vermin-proof cage. A particularly homogeneous plot of ground was selected for the experiment. The rows were arranged so that no given sample would be favoured by the position its rows occupied. The heated samples were separated from the controls as it was expected that several of these heated samples would give a low establishment percentage; had these rows been amongst the controls differential shading would have been unavoidable. It is believed that any (it must have been very small) differential shading between the control rows or the treated rows *per se* was compensated for by the chequer arrangement of the replications. The rows were spaced at a distance of one foot apart, 200 seeds were sown per row. Sowing took place on the 24th March.

Counts of the expanding first leaves were made at given intervals, the number of established plants was also estimated at frequent intervals. It was thought that data of value might be obtained by repeated inspection of the rows and the allocation of marks to each row upon a standard basis; such impressions as "general growth vigour" characterised by the amount of green leaf visible were regarded as a suitable basis for visual estimations. These inspection data were later carefully compared with absolute measurements including the number of plants, height and weight, and whereas they certainly conveyed a fairly good idea of the growth made they would not bear a detailed critical examination and therefore are not presented here.

As the experiment was designed primarily to trace out the effect of the seed characters upon early growth it was theoretically desirable, in the ideal case, to eliminate any other disturbing factors. In an attempt to meet this requirement it was decided to remove the plants for dry weight analysis before any competitive effects were apparent. Tillering, which does not take place to the same extent in every plant, begins very early at about the fourth week; it was thought that the effect of the seed characters would then still be apparent as indeed it has been demonstrated by other workers, Brenchley<sup>(1)</sup> and Findlay<sup>(3)</sup>, that size of seed influenced *final* yield in annual crops. The plants were removed on June 1st before any tillers had withered; all the plants were in the same "growth stage"; they were "leafy" and no tillers had shown any signs of panicle formation. Inequalities in actual size of the plants of different samples will be considered in the discussion of the data.

Table I.

*Showing the range of the figures used for the correlation coefficient calculations.*

(1) *Seed data.*

Weight per 1000 seeds gm.	% moisture content	Dry weight per 1000 caryopses gm.	% husk
Maximum 49.42	17.11	31.69	28.30
Minimum 30.42	12.52	19.05	23.47
Average 36.74	14.35	23.32	26.30

(2) *Sand test germination and field establishment data.*

Series	% germ. 4th day sand test	% total germ. sand test	% establish. seedlings, 3 weeks	% establish. plants, 10 weeks	Note
<i>Control</i>					
Maximum	87	98	38	37	* The poorest sample (Cc. 3069) included to provide an extreme case (see text).
Minimum	15*	24*	8*	10*	
Average	66	89	31	29	
<i>"Heated grain"</i>					
Maximum	81	97	38	34.5	
Minimum	2*	27*	8*	9*	
Average	43	88.5	29	26	

(3) *Growth data.*

Series	Dry weight of bulk obtained gm.	Average dry weight per 100 plants gm.
<i>Control</i>		
Maximum	262	82
Minimum	68	49
Average	179.5	65
<i>"Heated grain"</i>		
Maximum	285	84
Minimum	54	34
Average	152	57

Examination of the growing plants had proved that all, except one, of the 25 samples sown possessed the morphological characteristics of the type, *i.e.* Record, thus making the comparisons strictly legitimate.

It should be here pointed out that the dry weight of the top growth is here referred to as the "crop bulk" obtained. The roots were removed before drying, after the plants had been carefully counted. This bulk was obtained from 1000 seeds of each sample sown in rows and lifted ten weeks after sowing.

## V. THE ANALYSIS OF THE DATA BY CORRELATION STUDIES.

In order to ascertain the relationship between the seed characteristics and early growth, coefficients showing the degree or correlation between the fluctuating seed characters on the one hand, and the growth data on the other were calculated. Dot charts of the data were prepared and carefully examined; it is possible to obtain apparently significant mathematical coefficients for series of data owing to the position of one or two readings when the remainder of the pairs give dots on the charts grouped almost at random in a very small given area of the chart. The influence of these samples and the absence of any *general* correlation is revealed by the charts. Fisher<sup>(1)</sup> has shown "how deceptive in small samples is the use of the standard error of the correlation coefficient, on the assumption that it will be normally distributed." "Without this assumption the standard error [method] is without utility." For this reason the standard error, or probable error, method of obtaining the probability of the coefficient has not been employed. The probability of each correlation coefficient has been easily obtained by means of the specially prepared tables for a small number of "pairs" given by Fisher<sup>(1)</sup> and is shown in brackets after each coefficient. When the probability (*P*) is low the correlation is regarded as significant. Many of the coefficients calculated have of course been rejected. Table II shows the significant correlation coefficients and those of no mathematical significance mentioned in the text. A duplicate series of calculations in which the very poorest sample (Cc. 3069) has been omitted has been carried out. In this series there are 23 pairs. It is emphasised that these 23 samples are normal commercial samples of oats procured from the practical growers.

## V A. THE DIRECT CORRELATION COEFFICIENTS.

(1) *The relation between seed characteristics and germination.* When the dry weight per 1000 caryopses figures were plotted against those of the percentage husk it was found that there was no significant arrangement: the values of the coefficients are:

For 24 samples,  $R_{CL} = -\cdot277$  ( $P > \cdot1$ ).

For 23 samples,  $R_{CL}^1 = -\cdot162$  ( $P > \cdot1$ ).

That is to say, the average size of "kernel" was not related to the percentage husk in these samples. The value of the coefficients of correlation

<sup>1</sup> The index letters refer to the data of Table II, e.g. *C* represents the dry weight per 1000 caryopses, *L* represents the percentage of husk; see pp. 406 and 407.

Table II.

*The direct correlation coefficients obtained between seed characters and early growth data for 24 samples of Record oats showing the probability (P) of each coefficient. Calculations (omitting one special sample) are also shown for 23 samples.*

Weight per 1000 seeds	Refer- ence	Dry weight per 1000 caryopses	% germina- tion 4th day sand	% total germination sand	% seedlings established 3 weeks	% plants established 10 weeks	Total "bulk of crop," gm.	Average dry weight per 100 plants	% husk on seed
A	C	—	D (24) .190 P>.1	E (24) P>.1	F —	G (24) P>.1	H (24) .372 P<.1>.05	K (24) .513 P<.02	L —
			(23) 052 P>.1	(23) P>.1	—	(23) P>.1	(23) .372 P<.1>.05	(23) .535 P=.01	—
Moisture content of seed	B	(24) 039 P>.1 (23) .107 P>.1	(24) -.458 P=.02	(24) -.413 P=.05	(24) P>.1	(24) P>.1	(24) P>.1	—	—
			(23) -.427 P=.05	(23) -.473 P=.02	(23) P>.1	(23) P>.1	(23) P>.1	—	—
Dry weight per 1000 caryopses	C	—	(24) -.135 P>.1 (23) -.096 P>.1	(24) P>.1 (23) P>.1	(24) P>.1 (23) P>.1	(24) P>.1 (23) P>.1	(24) .388 P>.1 (23) .135 P>.1	(24) .538 P<.01 (23) .514 P<.02	(24) -.277 P>.1 (23) -.162 P>.1

D	% germination 4th day sand test	—	—	(24) .787 $P < .01$	(24) .480 $P < .02$	(24) .405 $P = .05$	(24) $P < .1$	(24) $P = .1$
				(23) 435 $P < .05$	(23) —	(23) $P > .1$	(23) $P > .1$	(23) $P > .1$
E	% total germination sand test	—	—	—	(24) —	(24) .724 $P < .01$	(24) .573 $P < .01$	(24) .383 $P < .01$
				—	(23) —	(23) .357 $P < .1 > .05$	(23) .280 $P > .1$	(23) $P > .1$
F	% seedlings established 3 weeks	—	—	—	—	(24) .966 $P < .01$	(24) .680 $P < .01$	(24) .522 $P = .01$
				—	—	(23) .809 $P < .01$	(23) .485 $P = .02$	(23) .186 $P > .1$
G	% plants established 10 weeks	—	—	—	—	—	(24) .713 $P < .01$	(24) .450 $P = .02$
				—	—	—	(23) .551 $P < .01$	(23) .085 $P > .1$
H	Total "bulk of crop"	—	—	—	—	—	(24) —	(24) —
				—	—	—	(23) .297 $P > .1$	(23) —



between the percentage germination in sand after four days and the percentage husk was not significant nor was any significant grouping found in the charts made:

$$\text{For 24 samples, } R_{DL} = .344 \quad (P = .1).$$

$$\text{For 23 samples, } R_{DL} = .067 \quad (P > .1).$$

Similarly the early seedling establishment estimations showed no significant relationship with the percentage husk:

$$\text{For 24 samples, } R_{FL} = .187 \quad (P > .1).$$

$$\text{For 23 samples, } R_{FL} = .153 \quad (P > .1).$$

The conclusion is therefore reached that in such commercial samples, inside one pure type—Record—the percentage husk bears no relationship to the germination of the samples.

No significant relationship was found between the size (weight estimation) of grain and sand germination—the values were as follows:

$$\text{For 24 samples, } R_{CD} = -.135 \quad (P > .1).$$

$$\text{For 23 samples, } R_{CD} = -.096 \quad (P > .1).$$

$$\text{For 24 samples, } R_{AD} = -.190 \quad (P > .1).$$

$$\text{For 23 samples, } R_{AD} = -.052 \quad (P > .1).$$

The soil germination figures also showed no significant relationship with estimations of the size of the grain.

The average size of the caryopsis was not found to be related to the moisture content of the seed sample as is shown by these equations:

$$\text{For 24 samples, } R_{BC} = .039 \quad (P > .1).$$

$$\text{For 23 samples, } R_{BC} = .107 \quad (P > .1).$$

The correlation between the moisture content of the seed sample and the germination counts on the fourth day was a negative one:

$$\text{For 24 samples, } R_{BD} = -.458 \quad (P = .02). \quad (1)$$

$$\text{For 23 samples, } R_{BD} = -.427 \quad (P = .05). \quad (1a)$$

The dot charts of the data indicated a general grouping of the results in a significant manner. When the total germination figures were considered along with the moisture content the equations obtained were:

$$\text{For 24 samples, } R_{BE} = -.413 \quad (P = .05). \quad (2)$$

$$\text{For 23 samples, } R_{BE} = -.473 \quad (P = .02). \quad (2a)$$

The dot charts plotted from these readings also tended to indicate significance in these results; when therefore all the four equations and the two charts are considered together it can be safely concluded from them that samples stored in a moist condition do not germinate as well as samples which (by reason of better harvesting conditions) contain less

water; and that for this effect to be observed the moisture content need not be very high.

(2) *The influence of the factors "weight of seed" and "number of plants" upon growth.* The dry weight per 1000 caryopses, which is a direct measure of the food available for the seedlings, has been found to be correlated with the dry weight of the plants produced:

$$\text{For 24 samples, } R_{CK} = .538 \quad (P < .01). \quad (3)$$

$$\text{For 23 samples, } R_{CK} = .514 \quad (P < .02). \quad (3 a)$$

When the total weight per 1000 seeds was considered the value of the coefficient was not quite so high in the case of all the readings but was slightly higher when 23 readings were used, thus:

$$\text{For 24 samples, } R_{AK} = .513 \quad (P < .02). \quad (4)$$

$$\text{For 23 samples, } R_{AK} = .535 \quad (P = .01). \quad (4 a)$$

These results are in close agreement with the work of Brenchley<sup>(1)</sup> and others who have studied the large and small seed question in other crops. Previous work with oats carried out by the present writers had given similar results. The influence of the seed weight upon the individual plants was thus clearly discernible at this growth stage; towards the end of tillering.

When the total "crop bulk" was considered the direct coefficient between weight of seed and total crop was found to be much lower and of very doubtful significance.

These values of the coefficients between weight of seed and total crop were as follows:

$$\text{For 24 samples, } R_{AH} = .372 \quad (P < .1 > .05). \quad (5)$$

$$\text{For 23 samples, } R_{AH} = .372 \quad (P < .1 > .05). \quad (5 a)$$

In the case of the *dry* weight of the seed data smaller values were obtained:

$$\text{For 24 samples, } R_{CH} = .288 \quad (P > .1). \quad (6)$$

$$\text{For 23 samples, } R_{CH} = .135 \quad (P > .1). \quad (6 a)$$

Such values are of no significance. The influence of the weight of seed upon the growth of the individual plant was not apparent when the total crop yield was considered. The factor "number of plants" contributing to the yield had also to be taken into consideration.

A high degree of correlation existed between the number of plants and the mass of the total crop:

$$\text{For 24 samples, } R_{GH} = .713 \quad (P < .01). \quad (7)$$

$$\text{For 23 samples, } R_{GH} = .551 \quad (P < .01). \quad (7 a)$$

When the sample giving very poor germination and establishment was omitted the value of the coefficient was decreased; amongst the remaining 23 readings the range in establishment figures was very narrow. Somewhat smaller values were obtained for the coefficients of correlation between the earlier seedling establishment estimates and total crop:

$$\text{For 24 samples, } R_{FH} = .680 \quad (P < .01). \quad (8)$$

$$\text{For 23 samples, } R_{FH} = .485 \quad (P = .02). \quad (8 a)$$

Again the influence of the one very poorly germinating sample was considerable. The very high value of "*R*" obtained between the number of plants estimated at the first leaf stage, and when the plants were removed, indicates that in the interval of eight weeks, when once the plants were established, little differential killing or dying off took place. There was also no irregular delayed germination. These coefficients were:

$$\text{For 24 samples, } R_{FQ} = .966 \quad (P < .01). \quad (9)$$

$$\text{For 23 samples, } R_{FQ} = .899 \quad (P < .01). \quad (9 a)$$

It is pointed out that in this experiment the plants were protected from vermin, and free from disease, and that under other conditions, and in a less favourable season, there can be little doubt that more "casualties" would have occurred in an irregular way. When the total sand germination figures were considered with those of the weight of the crop a significant value was only found for the 24 readings:

$$\text{For 24 samples, } R_{EH} = .573 \quad (P < .01). \quad (10)$$

$$\text{For 23 samples, } R_{EH} = .280 \quad (P > .1). \quad (10 a)$$

When only those samples whose germination total was greater than 85 per cent. were considered the value of the coefficient of correlation between the total germination figures and total crop bulk was practically zero—

$$\text{For "germination selected" 18 samples, } R_{EH} = .002 \quad (P > .1).$$

This indicates that the high value obtained in equation (8) was caused by the few poor germination samples, and that for practical purposes, under such good conditions of soil and climate, samples having a sand germination total of 85 per cent. were as good as those having a higher germinating capacity.

The correlations between the sand test germination figures, and the percentage of plants established as seedlings three weeks after sowing, are given by the following figures (some of which are not shown in Table II): 4th day germination figure and establishment:

$$\text{For 24 samples, } R_{DF} = .480 \quad (P < .02).$$

$$\text{For 23 samples, } R_{DF} \text{ is of no significance.}$$

The influence of the poorest (germination) sample is very pronounced in this case.

The 5th day germination figures and establishment correlations were:

For 24 samples,  $R = .677$  ( $P < .01$ ).

For 23 samples,  $R = .313$  ( $P > .1$ ).

The correlation between the total sand germination figures and the percentage of plants established in the field was higher:

For 24 samples,  $R_{EF} = .805$  ( $P < .01$ ). (11)

For 23 samples,  $R_{EF} = .519$  ( $P < .02$ ). (11 a)

The high correlation coefficient was again caused by the samples of poor germination. When the samples of poor total germination were eliminated and those with a total of over 85 per cent. only considered, there was again found to be no correlation with the establishment in this narrow range of germination. It is interesting to observe that the final germination figures are more closely correlated with establishment than are the earlier figures. In a previous paper<sup>(10)</sup> evidence collected tended to show that rapidity of germination influenced establishment; in the commercial samples here tested no support is obtained for that view. When "the mean germination time" (*i.e.* time taken for 50 per cent. of the viable seeds to germinate) was plotted against the seedling establishment figures it was seen that only in the extreme cases of very poor germination was the time required correlated (negatively) with the soil establishment. The mathematical coefficient obtained is of very doubtful significance  $R = -.313$  ( $P > .1$ ). It is here pointed out that in these "mean germination time" estimations the range of the time data was very narrow—the maximum was 4.0 days, the most rapid sample requiring 3.4 days. Unless the technique of sand germination tests is made more elaborate—which would probably reduce the number of possible tests—and subjected to many refinements it is not possible to obtain more accurate determinations of the mean germination time, as the intervals at which germination counts can be made are necessarily wide.

The value of the coefficients between total sand germination and final establishment was somewhat smaller than those previously considered in equations (9) and (9 a), where seedling establishment was considered:

For 24 samples,  $R_{EG} = .724$  ( $P < .01$ ). (12)

For 23 samples,  $R_{EG} = .357$  ( $P < .1 > .05$ ). (12 a)

For 18 selected samples with a high germination (of over 85 per cent.)

$R_{EG} = 0$  approx. (12 b).

In equation (12 *b*) the correlation between the number of plants established and the sand germination totals of samples germinating above 85 per cent. is shown to be practically zero. That is, for final establishment as for seedling establishment, a germination total of 85 per cent. in a sand test was as satisfactory as a higher figure under these growth conditions. It is apparent that over the wider ranges of germination totals (up to 85 per cent.), the sand tests afford reliable indications of the soil germination potentiality of the seed samples. Moreover, it is seen that the weight of produce obtained was, over the widest range of samples related closely to the number of plants established, and contributing to that total. When, however, comparisons were confined to samples of a high germination total no such correlations were found inside the narrower germination range of 85 to 100 per cent.

The relationship between the total "crop bulk" obtained and the size of the plant remains to be considered. The values obtained for this coefficient were too small to be of any significance:

$$\text{For 24 samples, } R_{HK} = -\cdot089 \quad (P > \cdot1). \quad (13)$$

$$\text{For 23 samples, } R_{HK} = \cdot297 \quad (P > \cdot1). \quad (13 a)$$

It is of interest, however, to note that the omission of the sample which germinated poorly has caused a considerable change from a minus to a plus value. It has been previously observed in equations (7) and (8 *a*) that the total crop bulk was closely related to the number of plants, so that the relationship between the number of plants and the weight of the plants must be examined, as possibly this would be an indication of the intensity of the competition between the plants. The equations are:

$$\text{For 24 samples, } R_{FK} = -\cdot522 \quad (P = \cdot01). \quad (14)$$

$$\text{For 23 samples, } R_{FK} = -\cdot186 \quad (P > \cdot1). \quad (14 a)$$

It is seen that for 23 readings the number of plants bears no relationship to the size of the plants at this stage; it must be noted here that the range in the number of plants was small. When the very poorest germinating sample was included there was a tendency for the weight of the plants to vary inversely as their number. It may be permissible, therefore, to conclude that in the extreme cases where germination was very low the size of the resultant plants was affected by the number. This would help to explain the change in sign of the coefficient in equations (13) and (13 *a*).

Table III.

Showing the partial correlation coefficients after eliminating one factor. The probability ( $P$ ) is also indicated.

Equation reference Nos.	Factors for correlation coefficients	Factor reference	Factor eliminated	Reference	Values of $R$ , 24 samples		Values of $R$ , 23 samples	
					Direct coefficient	Partial coefficient	Direct coefficient	Partial coefficient
(3) and (3 a)	Dry weight of caryopses	C	—	—	$R_{A\alpha} = .538$ ( $P < .01$ )	—	$R_{\alpha\alpha} = .514$ ( $P < .02$ )	—
(4) and (4 a)	Dry weight of plants	K	Establishment	G	—	$R_{K\alpha} = .537$ ( $P < .01$ )	—	$R_{K\alpha} = .513$ ( $P < .02$ )
(5) and (5 a)	Weight of seed	A	—	—	$R_{A\alpha} = .513$ ( $P < .02$ )	—	$R_{A\alpha} = .535$ ( $P = .01$ )	—
(6) and (6 a)	Dry weight of caryopses	K	Establishment	G	—	$R_{K\alpha} = .568$ ( $P < .01$ )	—	$R_{K\alpha} = .547$ ( $P < .01$ )
	Dry weight of crop	A	—	—	$R_{A\alpha} = .372$ ( $P < .1 > .05$ )	—	$R_{A\alpha} = .372$ ( $P < .1 > .05$ )	—
	Dry weight of caryopses	H	Establishment	G	—	$R_{H\alpha} = .542$ ( $P < .01$ )	—	$R_{H\alpha} = .388$ ( $P < .1$ )
	Dry weight of crop	C	—	—	$R_{C\alpha} = .288$ ( $P > .1$ )	—	$R_{C\alpha} = .135$ ( $P > .1$ )	—
	Dry weight of plants	H	Establishment	G	—	$R_{H\alpha} = .533$ ( $P < .01$ )	—	$R_{H\alpha} = .185$ ( $P > .1$ )
	Establishment	K	—	—	$R_{K\alpha} = .450$ ( $P = .02$ )	—	$R_{K\alpha} = -.085$ ( $P > .1$ )	—
	Establishment	G	Weight of seed	A	—	$R_{G\alpha} = -.531$ ( $P = .01$ )	—	$R_{G\alpha} = -.158$ ( $P > .1$ )
	Dry weight of plants	K	—	—	$R_{K\alpha} = .450$ ( $P = .02$ )	—	$R_{K\alpha} = -.085$ ( $P > .1$ )	—
	Establishment	G	Dry weight of caryopses	C	—	$R_{G\alpha} = -.463$ ( $P < .05$ )	—	$R_{G\alpha} = .078$ ( $P > .1$ )
	Establishment	C	—	—	$R_{C\alpha} = .713$ ( $P < .01$ )	—	$R_{C\alpha} = .551$ ( $P < .01$ )	—
	Dry weight of crop	H	Weight of seed	A	—	$R_{H\alpha} = .772$ ( $P < .01$ )	—	$R_{H\alpha} = .559$ ( $P < .01$ )
	Establishment	G	—	—	$R_{G\alpha} = .713$ ( $P < .01$ )	—	$R_{G\alpha} = .551$ ( $P < .01$ )	—
	Dry weight of crop	H	Dry weight of caryopses	C	—	$R_{H\alpha} = .784$ ( $P < .01$ )	—	$R_{H\alpha} = .561$ ( $P < .01$ )

## V B. PARTIAL CORRELATION COEFFICIENTS.

The direct relationship between the factors "number of plants," "weight of seed," and the weight of the resulting plants has been observed. In Table III the partial coefficients are shown after one factor has been eliminated.

By eliminating the "number of plants" (*i.e.* establishment) factor the coefficient of correlation between weight of seed and the weight of the resulting plants was increased, as is shown by these equations:

Direct coefficient for 24 samples,  $R_{AK} = .513$  ( $P < .02$ ). (4)

Direct coefficient for 23 samples,  $R_{AK} = .535$  ( $P = .01$ ). (4 *a*)

Partial coefficient for 24 samples,  $R_{AK.G} = .568$  ( $P < .01$ ). (4 *p*)

Partial coefficient for 23 samples,  $R_{AK.G} = .547$  ( $P < .01$ ). (4 *ap*)

The values of the partial correlation coefficients between the *dry* weight of the caryopses and the resulting plants obtained after eliminating the establishment factor are as follows:

Direct coefficient for 24 samples,  $R_{CK} = .538$  ( $P < .01$ ). (3)

Direct coefficient for 23 samples,  $R_{CK} = .514$  ( $P < .02$ ). (3 *a*)

Partial coefficient for 24 samples,  $R_{CK.G} = .537$  ( $P < .01$ ). (3 *p*)

Partial coefficient for 23 samples,  $R_{CK.G} = .513$  ( $P < .02$ ). (3 *ap*)

The establishment factor was also eliminated from the correlation between the weights of the seed and the total "crop bulk" obtained, thus:

Direct coefficient for 24 samples,  $R_{AH} = .372$  ( $P < .1 > .05$ ). (5)

Direct coefficient for 23 samples,  $R_{AH} = .372$  ( $P < .1 > .05$ ). (5 *a*)

Partial coefficient for 24 samples,  $R_{AH.G} = .542$  ( $P > .01$ ). (5 *p*)

Partial coefficient for 23 samples,  $R_{AH.G} = .388$  ( $P > .1$ ). (5 *ap*)

When the value of the coefficient of correlation between the *dry* weight of the caryopses and the total "crop bulk" were considered the effect of eliminating the establishment factor was found to be an increase in the value of "*R*." The probability figure was too high for equation (15 *ap*) to be of significance:

Direct coefficient for 24 samples,  $R_{CH} = .228$  ( $P > .1$ ). (15)

Direct coefficient for 23 samples,  $R_{CH} = .135$  ( $P > .1$ ). (15 *a*)

Partial coefficient for 24 samples,  $R_{CH.G} = .533$  ( $P < .01$ ). (15 *p*)

Partial coefficient for 23 samples,  $R_{CH.G} = .185$  ( $P > .1$ ). (15 *ap*)

These values were obtained when the more accurate measurements of the food reserves in the seed, the *dry* weight of the caryopses, were employed. The elimination of the establishment figures from the

equation for 24 samples has in both cases (*dry* weight of caryopses and seed weight, in equations (5 *p*) and (15 *p*) respectively,) given a significant coefficient with the crop bulk. In the 23 normal samples it is concluded from equations (5 *ap*) and (15 *ap*) that the weight of the seed was not closely correlated with the weight of the crop produced.

The correlation between the weight of the plants and the number of plants (establishment) is of no significance for the 23 samples, but of apparent significance for the 24 samples due to influence of the one sample of exceptionally poor germination. When the weight of the seed (*A*) and the *dry* weight of the caryopses (*C*) were in turn eliminated the values of the partial correlation coefficient between the number of plants and the size of the plants were of no significance. The actual values obtained are as follows:

Direct correlation for 24 samples,  $R_{KG} = -0.450$  ( $P = .02$ ). (16)

Direct correlation for 23 samples,  $R_{KG} = -0.085$  ( $P > .1$ ). (16 *a*)

Partial correlation for 24 samples,  $R_{KG.A} = -0.531$  ( $P = .01$ ).

Partial correlation for 23 samples,  $R_{KG.A} = -0.158$  ( $P > .1$ ).

where the "weight of seed" factor has been eliminated.

Partial correlation for 24 samples,  $R_{KG.C} = -0.463$  ( $P < .05$ ).

Partial correlation for 23 samples,  $R_{KG.C} = -0.078$  ( $P > .1$ ).

where the "dry weight of caryopses" factor has been eliminated.

The random distribution of the points on the dot chart indicated that there was no relation between the number of plants and their weight at this particular growth stage, when a narrow range of normal establishment figures was considered (see (16 *a*)). When the sample of very poor germination was included the greatly increased space per plant has caused the plants to be larger and a negative value of *R* was obtained (see (16)).

The weight of the "crop" has been shown to be closely correlated with the number of plants (equation (7)); when the effect of the weight of seed (*A*) and the *dry* weight of the caryopses (*C*) were eliminated the values obtained were increased:

Direct coefficient for 24 samples,  $R_{GH} = 0.713$  ( $P < .01$ ). (7)

Direct coefficient for 23 samples,  $R_{GH} = 0.551$  ( $P < .01$ ). (7 *a*)

Partial coefficient for 24 samples,  $R_{GH.A} = 0.772$  ( $P < .01$ ).

Partial coefficient for 23 samples,  $R_{GH.A} = 0.559$  ( $P < .01$ ).

where the "weight of seed" is eliminated.

Partial coefficient for 24 samples,  $R_{GH.C} = 0.784$  ( $P < .01$ ).

Partial coefficient for 23 samples,  $R_{GH.C} = 0.561$  ( $P < .01$ ).

where the "dry weight of caryopses" is eliminated.



## VI. THE EFFECT OF HEATING THE SEED.

The seed samples were heated in a large drying oven for 15 minutes at 75° C. The seed was spread out so that the grain quickly acquired the oven temperature. The effect of this treatment upon germination and field establishment and growth was observed. As the heated samples of grain were not sown in the rows spaced amongst the control samples the soil growth and yield data of the two series were not critically compared. These samples, therefore, constituted a second series and correlation studies were therefore only of value within the series. The *general* effect of this short exposure to high temperature is shown in the following table:

	Control series	Treated series
Average 4th day sand germination ... ..	68 %	43.08 %
Average of "mean germination period*" ... ..	3.62 days	4.04 days
Average total germination ... ..	89 %	88.5 %
Average final field establishment ... ..	28 %	26.5 %
Average weight per 100 plants ... ..	65.1 gm.	57.2 gm.
Average total yield "per space unit" from 1000 seeds sown ... ..	182 gm.	152 gm.

\* "Mean germination period" = time required for the germination of 50 % of the viable seeds of a given sample.

The heating treatment has retarded the rate of germination and reduced the number of plants established and has also decreased the average weight per 100 plants. These results are similar to those obtained by Groves (5), who exposed grain to high temperatures for varying periods.

## VII. CORRELATION STUDIES.

A positive value, but of doubtful significance for the correlation coefficient between the moisture content of the seed and the reduction in the 4th day germination figure produced by heating was obtained.

For all the samples  $R = .368$  ( $P < .1 > .05$ ).

Other workers have also shown that the moisture content of the seed is related to the injury that occurs when samples of grains are heated.

That some protection was afforded by the husk was shown by the correlation coefficient between the reduction in the 4th day germination figures and the per cent. husk, the value of which was

$R = -.370$  ( $P < .1 > .05$ ).

It would appear that the heavier seeds were more liable to injury by this treatment, for the correlation between seed-weight and the reduction in the number of the seeds germinating was positive.

Coefficient of correlation between weight per 1000 seeds and decrease in the 4th day germination figure,

$$\text{For 24 samples, } R = .427 \quad (P < .05).$$

Coefficient of correlation between *dry* weight per 1000 caryopses and decrease in 4th day germination figure,

$$\text{For 24 samples, } R = .415 \quad (P < .05).$$

The samples that germinate quickly (a high 4th day germination figure) were more susceptible to the injurious effect of the heating than samples germinating more slowly. The coefficient of correlation between 4th day germination figures of control samples and the decrease in the 4th day germination produced by heating the grain was:

$$\text{For 23 samples, } R = .411 \quad (P = .05).$$

It has been observed that the magnitude of the injurious effect of heating the grain was correlated with the size of the grain and also with the rate of germination of the sample under normal conditions. The weight of grain influenced the growth and weights of the control series of plants as shown by the earlier data in this paper. It was therefore thought that a positive correlation would exist between the magnitude of the injurious effect of heating as shown by germination figures, and the weights per 100 plants of the unheated (control) series. When calculated it was found to be of no significance, however,

$$R = .322 \quad (P > .1).$$

The relationship between the germination figures and establishment is shown by the following coefficients for the heated grain series:

Fourth day sand germination and seedling establishment in soil

$$R = .063 \quad (P > .1).$$

Fifth day sand germination and seedling establishment in soil

$$R = .327 \quad (P > .1).$$

Total sand germination and seedling establishment in soil

$$R = .535 \quad (P < .01).$$

If, however, only those samples of a germination of more than 85 per cent. were considered there was no correlation between total germination and establishment. The correlation between the early seedling establishment figures and the final establishment of larger plants was again high,

$$R = .889 \quad (P < .01).$$

The total "crop bulk" was closely related to "the number of plants" factor; the value for the series of this coefficient is

$$R = .731 \quad (P < .01).$$

In the control series the dry weight of the plants was found to be correlated with the weight of the seed and the amount of available food in the caryopsis. In the heated series the coefficient was of no material significance,

$$R = .252 \quad (P > .1).$$

It would therefore appear that the heating had directly affected the availability of the food supply (or that an unforeseen disturbing factor had influenced the dry weights of these plants). The magnitude of the difference between the dry weight of the control plants and the dry weight of the plants from heated grain, gave too small a positive correlation

$$R = .296 \quad (P > .1)$$

with the moisture content of the grain to show any tendency that most grain suffers most from this treatment, from this growth point of view (for germination effect see p. 416).

This treatment, heating the grain, influenced the "yield" by operating upon the "number of plants" factor. This was shown by the high value of the correlation coefficient between the decrease in the number of plants caused by heating and the decrease in the total bulk of the crop produced. These differences from the control figures were closely correlated

$$R = .728 \quad (P < .01).$$

As a result of the preliminary work reported in a previous paper it was hoped that heating the grain and observing the effect upon germination might serve as a practical method of testing a sample's capabilities. Briefly, this extended trial has shown that a high moisture content is correlated with poor germination, and that such samples are probably more easily injured by the heating treatment. A "lively" quickly germinating sample was, however, susceptible to this injury; the heavy samples of high germination capacity have been also found to be more liable to injury. So that whilst further information can be obtained from the heating treatment it does not alone serve as a method of distinguishing undesirable samples.

#### VIII. GENERAL DISCUSSION.

There can be little doubt that there are two factors, contemporary for at least part of the growth period, influencing the size of the individual plants; these are the weight of the seed and the establishment (or germination total) factor. The influence of the seed is such that heavy seeds tend to produce large plants, whilst the influence of establishment, more clearly seen in the extreme cases, is such that low

germination figures tend to produce heavy plants. There is no evidence to show that these two factors operate one upon the other, that is, that heavy seeds necessarily germinate well; for it has been demonstrated that the correlation coefficients between the weight of the seed and the germination figures are of no significance. An attempt has been made to evaluate the influence of these two factors upon the growth of the individual plants and upon the total "crop bulk" obtained when estimated at this particular growth stage<sup>1</sup>. It is, of course, not assumed that the relative intensity of these two factors is constant for the entire growth period, or that they operate for an equal period; it is to be regretted that no oat data are available from which estimations of the degree of correlation between the size of seed and establishment on the one hand and growth on the other hand could be ascertained for any other growth stage than maturity (*i.e.* harvest data). The data here presented only show one point in the whole growth cycle. From *a priori* considerations it might be supposed that the weight of the seed factor would influence early growth more particularly and that the spacing factor, "establishment," would tend to show its maximum effect at a later developmental stage.

The data presented in this paper show that the weight of the plant is closely correlated with the weight of the seed, but when the whole crop bulk is considered the "number of plants," dependent upon germination and establishment, is the more closely correlated factor. Only in extreme cases does the additional space provided by very poor germination cause the weight of the plants to be increased, with normal germination and space this compensating power, a function of tillering is not revealed. Working with barley Engledow and Wadham (2) state that as a general rule the first formed is the largest and heaviest tiller so that  $T_0 > T_1 > T_2 \dots$ , this is, in the opinion of the writers, applicable also to oats; so that the importance of a large number of plants each contributing one large tiller is emphasised. This conclusion is in agreement with previous work carried out by the writers with oats, as a result of which they emphasised the importance of establishment as a factor influencing yield in spring-sown oats in Wales.

Recently Pettinger (9) conducted a series of experiments to study the relationship of seedling characters and final yield. He measured the coleoptiles of the germinating seeds, but considered that other factors besides the amount of food stored influenced coleoptile length; he, however, fully realised that the seed weight influenced early growth in oats

<sup>1</sup> Tiller production was almost if not entirely completed; no small tillers had begun to wither, no signs of panicle production were observed.

and for his experiments used representative samples including large and small grain. No correlation of biological significance was found between coleoptile length and yield in the "Early Champion" material he employed. Other workers who have searched for an index of yield have compared various genetical types, but generally it may be said that no satisfactory index of yield has been found. Newman<sup>(8)</sup> reviews the Svalöf work from this point of view.

The data here discussed were obtained from Record oats which is believed to be genetically "pure." Inside such a variety samples of seed have been tested and the vigour of their early growth estimated. It has been shown that heavy or plump seed produce larger seedlings and that the number of plants determines the bulk of crop produced at one particular growth stage. It is not suggested that a similar result would be obtained with an exceptionally heavy tillering variety as "grey winter" or a "late" spring variety, but in this variety—Record—compensation for germination irregularities is not complete by any means when tiller initiation is completed. The importance therefore of a high percentage establishment is again emphasised.

#### SUMMARY.

A series of correlation studies has been carried out between the seed characteristics and early growth data of a number of Record oats samples; many coefficients of correlation obtained gave values of no biological significance. The influence of "outlying" readings had to be carefully considered as the number of readings was limited. For this purpose dot charts were employed as well as mathematical coefficients.

The results can be summarised as follows:

1. Generally the total germination figures in a sand test were correlated with field establishment, but within the narrow germination range 85–100 per cent. no correlation with establishment was found.

2. The amount of available food in the seed as measured by the average dry weight of the caryopses influenced the size and weight of the seedlings produced.

3. The moisture content of the grain was negatively correlated with the rate of germination, indicating that samples stored with a high water content did not germinate well.

4. The percentage husk bore no relation to germination.

5. Ten weeks after sowing the mass of the plants obtained from a given sowing depended primarily upon the number of plants established. Although tillering was well advanced plants possessing additional space

did not compensate for low establishment figures at this stage by increased growth.

6. When the grain was heated for 15 minutes at 75° C. those samples containing a high moisture content suffered more than well dried samples. Indications were also obtained that the following type of sample was particularly susceptible to such injury—artificial heating—

- (a) a rapidly germinating sample,
- (b) a thin-husked sample,
- (c) a sample with heavy grain.

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#### LITERATURE CITED.

- (1) BRENCHEY, W. E. (1923). Effect of the Weight of Seed upon the Resulting Crop. *Ann. App. Biol.* x, No. 2, p. 223.
- (2) ENGLEDOW, R. L. and WADHAM, S. (1923 *et sub.*). Investigations on Yield in the Cereals. *Journ. Agric. Sci.* xiii, p. 390; xiv, pt. 1, p. 66; xiv, pt. 2, p. 287, and xiv, pt. 3, p. 325.
- (3) FINDLAY, W. M. (1919). The Size of Seed. *N. of Scot. Coll. of Agric. Bul.* No. 23.
- (4) FISHER, R. A. (1925). *Statistical Methods for Research Workers*. Oliver and Boyd, London.
- (5) GROVES, J. F. (1917). Temperature and Life Duration of Seeds. *Bot. Gaz.* LXIII, p. 169.
- (6) JONES, MARTIN G. (1923). Preliminary Investigations with Oats. *Welsh Plant Breeding Station Bul.* Series C. No. III, p. 19. Univ. Coll. of Wales.
- (7) JONES, MARTIN G. and TINCKER, M. A. H. (1926). Yield Studies in Oats. The Effect of the Pre-treatment of the Parent Crop upon the Seed Produced, its Germination and Subsequent Growth. *Ann. App. Biol.* xiii, No. 4, p. 535.
- (8) NEWMAN, L. H. (1912). Plant Breeding in Scandinavia. *The Canadian Seed Growers' Ass.*, Ottawa.
- (9) PETTINGER, N. A. (1925). The Relation of Coleoptile Length to Yield in Oats. *Journ. Amer. Soc. Agron.* xvii, No. 9, p. 537.
- (10) TINCKER, M. A. H. (1925). Physiological Predetermination Experiments with Certain Economic Crops. The Relation Between Rate of Germination and Subsequent Growth. *Ann. App. Biol.* xii, No. 4, p. 440.

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## SULPHUR TREATMENT OF SOIL AND THE CONTROL OF WART DISEASE OF POTATOES IN POT EXPERIMENTS

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(With 1 Text-figure.)

FIELD experiments described elsewhere<sup>(2)</sup> on potatoes grown in soil heavily contaminated with *Synchytrium endobioticum*, the fungus causing Wart Disease, have shown that thorough incorporation of finely divided sulphur with the soil may be followed by an almost clean crop of potatoes while neighbouring untreated plots give heavily infected plants. Since sulphur is oxidised to sulphuric acid in the soil, the treatment necessarily leads to an increased acidity except in those soils which contain large reserves of calcium carbonate. The question whether the toxicity of sulphur depends entirely on the development of a high degree of acidity or whether some other factor takes part has practical importance as well as theoretical interest. If a large increase in the soil acidity is necessary, the treatment will be impracticable owing to the high initial cost and the harmful effects on succeeding crops; if however the sulphur has some other mode of action, there remains the possibility of increasing its efficiency and of minimising the ill effects on later crops.

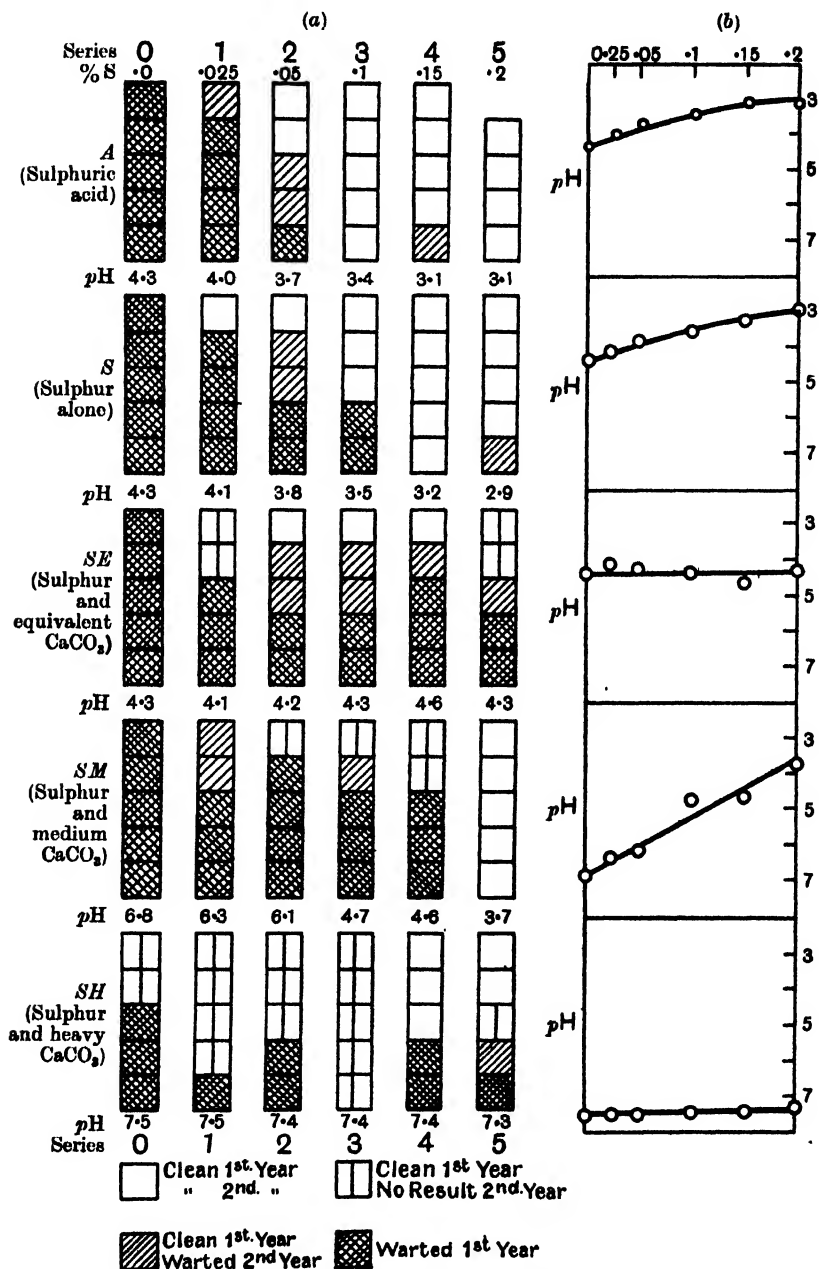
Evidence from field experiments has already been advanced to indicate that the toxic action is not a simple function of the final soil reaction<sup>(2)</sup>. Mention was also made in this paper of a series of pot experiments which was planned to discriminate between two possible modes of action of sulphur, but which failed owing to the absence of infection in the crop grown. This experiment has now been carried out successfully and has afforded some further evidence in support of the hypothesis of a dual mechanism of sulphur toxicity.

## EXPERIMENTAL.

The tests were carried out on potatoes grown in pots in an acid soil from Stalybridge, Cheshire, with which winter sporangia of the fungus were mixed. Each of five series of treatments consisted of 30 pots giving fivefold replicates of six different quantities of sulphur (0, .025, .05, .1, .15 and .2 per cent. on the air-dry soil). In series *A* (Text-Fig. 1) the sulphur was added in the form of sulphuric acid in order to eliminate as far as possible the effects of intermediate compounds of sulphur. All the remaining series received finely ground sulphur, series *S* having sulphur only. In series *SE* the sulphur was accompanied by an equivalent amount of calcium carbonate to maintain an approximately constant final reaction so as to test the effects of possible intermediate compounds. A constant dressing of calcium carbonate was given to each pot in the *SM* series in an amount sufficient to bring the soil without sulphur almost to the neutral point. This dressing was equivalent to 0.15 per cent. sulphur so that treatments *SE* 4 and *SM* 4 were identical and were represented by a single set of 5 pots. Treatments *SM* 0 to *SM* 3 had an excess of calcium carbonate over sulphur, whilst treatment *SM* 5 had an excess of sulphur. Finally, in series *SH* a large excess of calcium carbonate (5 per cent.) was used so that a considerable amount remained and gave a slightly alkaline reaction even with the heaviest dressing of sulphur.

For each treatment 15 lbs. of soil, passing a 3 mm. sieve, were mixed thoroughly with 60 c.c. of a fine dry powder consisting of decayed warts and then with the appropriate chemicals; the additions and incorporation were carried out with the utmost care so as to ensure uniform distribution. Each 15 lb. lot of soil was divided equally between five ordinary flower pots and the whole series kept in a moist state. After four weeks one tuber of the highly susceptible variety, Arran Chief, was planted in each pot and the soil kept fairly moist for a further month, by which time shoots had appeared above the surface. From this time the soils were maintained in a very wet condition in order to favour infection(1). The soil proved to be difficult to wet, especially in the more acid series, and growth was so slow that it was considered advisable to allow five months after planting before lifting. By this time fairly good growth had taken place under each treatment. The pots which had given uninfected plants were kept in an air-dry state throughout the winter and in most cases a second crop of potatoes was grown in the following summer.





Each square represents the result obtained in one pot.

Fig. 1. (a) Showing soil treatment, soil reaction and infection. (b) Showing percentage sulphur and soil reaction.

The numbers of clean and warted plants obtained after each treatment are set out diagrammatically in Fig. 1. Where for any reason as in most of the SH. series the growth in the second season was not sufficient to afford an adequate chance of infection, "no result" is recorded in the diagram. Electrometric determinations of the  $pH$  values of the soil were made after the first month and again after removing the first crops. These showed that the oxidation of the sulphur was almost complete in one month in all but the heaviest dressings. The  $pH$  values obtained when the crops were removed are entered under each column in Fig. 1*a*, and for ease of visual comparison are plotted in Fig. 1*b*, as a function of the amount of sulphur added.

#### DISCUSSION.

The method adopted proved successful. Treatments A. 0, S. 0, SE. 0, in Fig. 1 represent 15 independent untreated pots every one of which gave an infected plant in the first year. With several of the treatments each of the five replicate pots gave uninfected plants in two seasons. The remaining treatments show a steady gradation between these extremes.

Sulphur (S.) and sulphuric acid (A.) have similar effects in reducing infection and in increasing the acidity. 0.1 per cent. sulphur as sulphuric acid and 0.15 per cent. sulphur as element and heavier dressings of either gave complete freedom from disease in the first year, and only 2 pots out of 21 became infected in the second year. This striking reduction of infection occurred when the acidity had exceeded that represented by  $pH$  3.4, a degree of acidity somewhat higher than that put forward by Weiss(3) as the maximum acidity at which wart disease occurs viz.  $pH$  3.9. This degree of acidity is considerably greater than that found in fertile arable soils, and there would be no opportunity of utilising sulphur as a practical soil treatment if its efficacy depended entirely on the development of an acidity exceeding some such limiting value.

An indication that sulphur reduces infection under conditions which preclude the production of such extreme acidities and which are thus more comparable with the field results is given by certain treatments in the present experiment. Thus in the SE. series the reaction in all cases is substantially the same as in the untreated soils, but half of the pots gave clean plants in the first year whilst all of the 14 untreated pots gave infected plants. Again in the series with a constant medium dressing of calcium carbonate, treatments SM. 1, SM. 2, and SM. 3 gave some

clean plants in the first year in soils which were less acid than the untreated soil. In the absence of sulphur this amount of calcium carbonate did not reduce infection. In SM. 5, with 0.2 per cent. of sulphur and calcium carbonate equivalent to 0.15 per cent. sulphur, there was no infection in either season although the final reaction approximated to that given by 0.05 per cent. sulphur, either as element or as sulphuric acid, and was less acid than that given by 0.1 per cent. sulphur as element. These smaller sulphur dressings however allowed appreciable infection.

In the presence of a large excess of calcium carbonate there was little infection in the first year, and even in the absence of sulphur two out of five pots gave clean plants. In an earlier experiment on potatoes grown in an artificially infected peat a few clean plants were obtained when very heavy dressings of calcium carbonate were applied. These results are in harmony with those of Weiss(3) in whose experiments infection was considerably reduced when the pH was 7.5 or more. Attention may also be drawn to the fact that wart disease appears to be more prevalent in the acid soils of semi-industrial areas than in the calcareous soils.

No explanation can be afforded at present for the absence of any connection between the extent of infection and the amount of sulphur in Series SE., SM. (excepting SM. 5) and SH.

#### SUMMARY

In a series of pot experiments on potatoes grown in an acid soil artificially infected with the wart disease fungus, treatments with sulphuric acid and various combinations of sulphur and calcium carbonate, yielding a wide range of soil reaction, gave almost complete freedom from infection when the acidity of the soil had been raised to a very high value (pH 3.4 or less).

Heavy dressings of calcium carbonate, alone or with sulphur, giving a soil reaction of pH 7.5 or more, also reduced infection.

The fact that partial and even, in one experiment, complete suppression of disease was obtained at lower acidities, where the effect on the disease was not closely related to the degree of acidity, supports the tentative conclusion already drawn from field experiments that sulphur in controlling wart disease does not depend entirely on its effect in raising the acidity, but has also some other mode of action. Whether this toxicity which sulphur exerts apart from its effect on the acidity can be enhanced sufficiently to be of any practical value requires further investigation.

REFERENCES.

- (1) GLYNNE, MARY D. (1925). Infection Experiments with Wart Disease of Potatoes. *Ann. App. Biol.* XII, pp. 34-60.
- (2) ROACH, W. A., GLYNNE, M. D., BRIERLEY, W. B. and CROWTHER, E. M. (1925). Experiments on the Control of Wart Disease of Potatoes by Soil Treatment with particular reference to the use of Sulphur. *Ann. App. Biol.* XII, pp. 152-190.
- (3) WEISS, FREEMAN. (1925). The Conditions of Infection of Potato Wart. *Amer. Journ. Bot.* XII, pp. 413-442.

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## THE EFFECT OF HYDROGEN PEROXIDE ON YEAST GROWTH AND FERMENTATION

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(With 2 Text-figures.)

YEAST, with its accompanying enzymes, produces a vigorous fermentation under anaerobic conditions but multiplication of the yeast cells is greatly inhibited under these circumstances. Successful commercial propagation of yeast is attended by heavy aeration, by which means as high as 40 per cent. of the weight of nutrient used in the mash has been converted into yeast cells<sup>(1)</sup>. A review of the literature dealing with the effects of aeration and other gases on yeast growth and fermentation has been given previously<sup>(2, 3)</sup>. All of these former investigations were carried out under conditions which were essentially those most favourable for fermentation, rather than those which are best for rapid increase of yeast crop. The high yields of yeast under the favourable influence of heavy aeration may be due partly to the fact that yeast growth is aerobic in character. In this case, the optimum conditions would be those involving maximum interface between the gas and the mash. Aeration is also an efficient means of stirring whereby the yeast cells come into more intimate contact with their food. In studying the accelerative effect of aeration, it occurred to us that if the concentration of oxygen in the mash were the critical factor, it might be possible to produce the same acceleration by the gradual addition of hydrogen peroxide, at the same time substituting the mechanical effect of aeration by vigorous stirring. Hydrogen peroxide is rapidly decomposed by the catalase of the yeast with the formation of molecular oxygen and water, as is evidenced by the fact that if a suspension of yeast is added to 3 per cent. peroxide a rapid evolution of oxygen takes place. Our experiments show no favourable effect from the nascent oxygen thus introduced into the mash but demonstrate that the peroxide is exceedingly toxic, both to the yeast and to its accompanying enzymes. However, this toxicity

is only temporary, since yeast growth and fermentation proceed in a normal fashion after the peroxide has been removed by the action of the catalase.

Our experiments also compare the chemical changes and growth with and without aeration and confirm the accelerative effect of aeration reported by other investigators.

#### EXPERIMENTAL PART.

The general procedure in this series of experiments was the same as that described previously by one of us and A. K. Balls(2, 3). However, in the experiments described here only the principal chemical reactions taking place in the mash, such as changes in total solids, total sugar, rate of inversion (reducing sugar), yeast crop and pH were followed. These were determined exactly as before(2, 3). The yeast crop determination should be especially mentioned, however, since the actual dry weight of the yeast is recorded rather than the cell count which has been the usual index of yeast growth employed by others. It should also be noted that the total sugar is estimated as invert sugar and since it is present at the start chiefly as sucrose, the results reported for total sugar for the zero and second hours are too high by something less than 6 per cent.

At the beginning of each experiment, the various constituents of the mash were placed in a large specimen jar and thoroughly stirred by air introduced from a block tin pipe perforated with fine holes. A known quantity of yeast<sup>1</sup>, in aqueous suspension, was then added and the contents of the flask diluted with water to the desired volume. To avoid errors due to evaporation, the air was first passed through two wash bottles to saturate it with water. All of the apparatus was kept in a thermostat at 28-28.5° C. After stirring for two minutes, the first samples were removed for analysis. The analysis was repeated after two hours; and after about four hours (the exact time is noted in each experiment) the mash was divided into three portions. The first part (A) served merely as a control and was untreated, aeration being continued as before. The second portion (B) consisted of a litre of mash, aerated in the same manner as the control, but treated with 30 per cent. hydrogen peroxide<sup>2</sup>. This addition was made very slowly at first, three cubic centimetres being added over a two hour period (fourth to sixth

<sup>1</sup> The yeast which was employed in these experiments was fresh baker's yeast, *Saccharomyces cerevisiae*, which was kindly furnished us by the Fleischmann Co.

<sup>2</sup> Superoxol, Merck.

Table I.  
*Experimental Data on Mashcs.*

Hour	Total sugar			Reducing sugar			Total solids			Yeast $\alpha$			pH		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Experiment I															
0	24.00	—	—	—	—	—	27.47	—	—	1.03	—	—	7.0	—	—
2	23.69	—	—	—	—	—	27.00	—	—	1.30	—	—	6.5	—	—
3.75	17.88	—	—	—	—	—	24.89	—	—	2.26	—	—	5.5	—	—
5.75	6.46	—	9.91	—	—	—	14.36	24.42	17.24	4.81	2.53	3.58	3.0†	4.6†	4.0†
8	1.77	15.92	0.83	—	—	—	9.95	24.37	8.87	7.00	2.60	4.60	6.5	6.5	6.5
26	0.55	16.64	0.48	—	—	—	9.91	23.45	8.68	8.20	2.66	4.04	6.2	6.2	6.2
Experiment II															
0	28.96	—	—	2.65	—	—	31.36	—	—	1.27	—	—	7.0	—	—
2	28.12	—	—	16.17	—	—	31.02	—	—	1.70	—	—	6.8	—	—
3.5	24.40	—	—	20.62	—	—	28.53	—	—	2.42	—	—	6.3	—	—
6.0	13.36	21.68	13.97	11.26	18.74	12.18	16.40	23.68	19.07	7.38	3.17	3.43	3.4†	5.0	5.0
8.0	0.77	22.00	2.14	0.41	19.13	1.61	10.00	23.40	10.68	7.74*	3.32*	4.18*	—	—	—
26	0.38	0.40	0.93	—	0.40	—	9.52	8.88	8.18	9.27†	6.72†	—	6.0	4.8	4.8

\* Analysis at 7.5 hour.

† Analysis at 25 hour.

‡ Raised to pH 6.5 with  $\text{NaHCO}_3$ .

$\alpha$  Microscopical examination of the yeast was made at the time of each analysis to observe the general appearance of the yeast. No important infections were noted.

hour), while an additional 47 c.c. were added between the sixth and eighth hour. The third part (C) consisted of about 750 c.c. of the mash stirred without aeration, except for such air as unavoidably came into contact with the surface. Further analyses were made at the fourth, sixth, eighth and twenty-sixth hours. Fermentation in the control was practically complete at the end of the eighth hour.

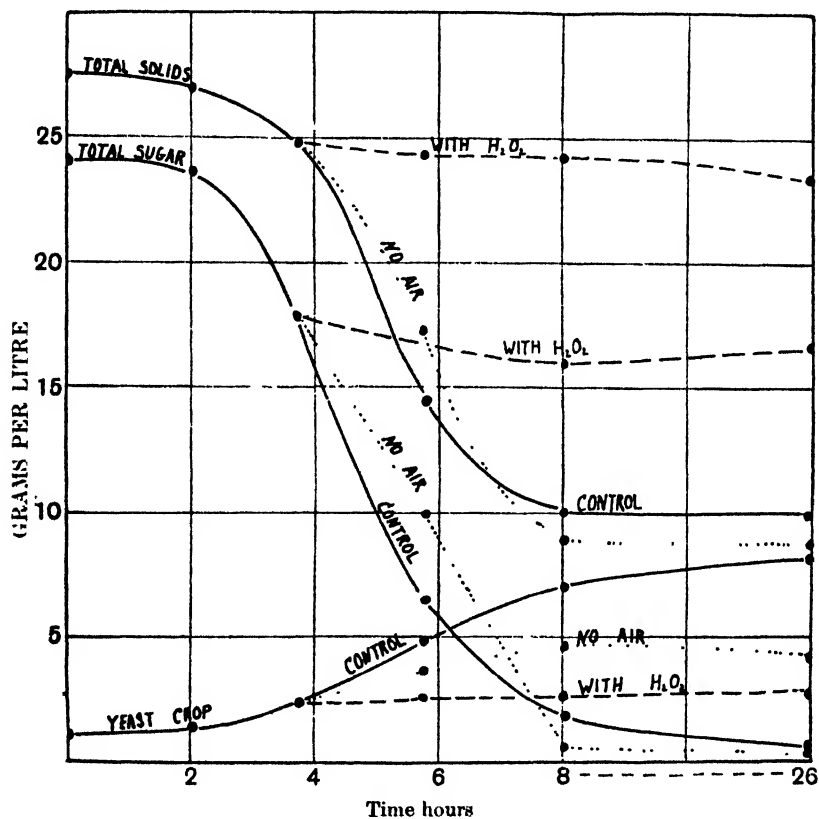


Fig. 1.

Only two typical experiments will be described here. Several trials similar to these two were carried out with like results, and it is unnecessary to show duplicate data.

*Experiment I.* Three litres of mash were made up as follows: molasses, 16; sucrose, 24;  $(\text{NH}_4)_2\text{HPO}_4$ , 1.2;  $(\text{NH}_4)_2\text{SO}_4$ , 1.6; yeast, 2.8 (all weights in grams per litre). At the beginning of the experiment the pH was adjusted to 7.0 by the addition of a few drops of sulphuric acid. Later on, when the pH had fallen, it was raised to 6.5 by the addition of small



amounts of sodium bicarbonate. The division into three mashes was made at the end of  $3\frac{3}{4}$  hours. Lot one of the superoxol was used here. Results in Table I and Fig. 1.

*Experiment II.* This was an exact duplicate of (1) except that lot two of the superoxol was employed, and also that the second addition

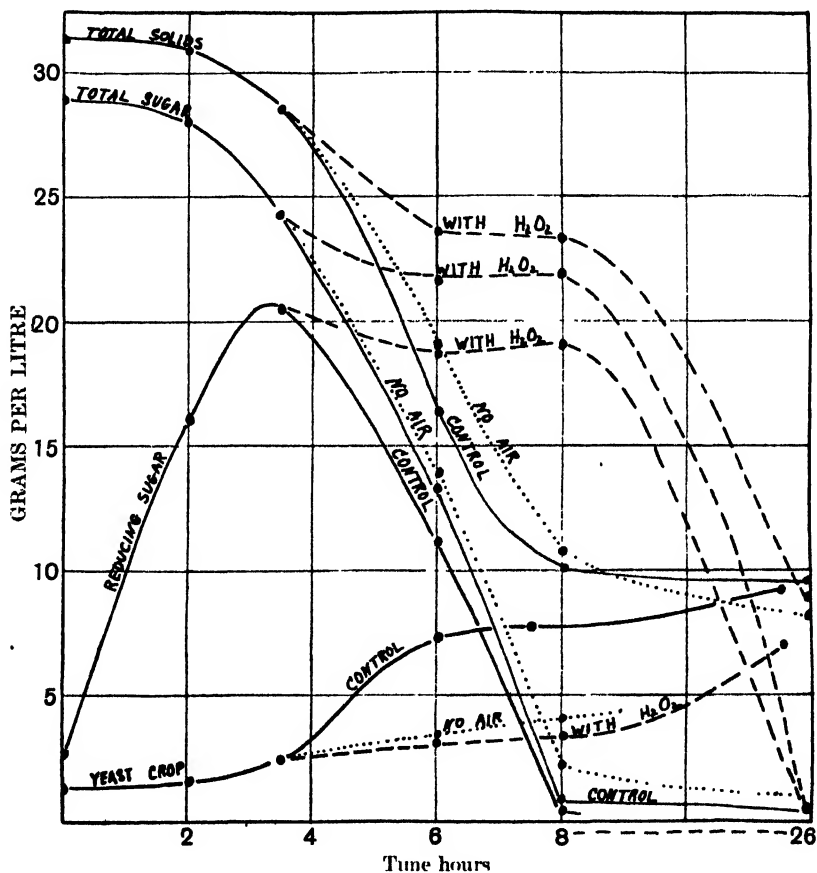


Fig. 2.

of superoxol was made in one portion of 47 c.c. at the sixth hour. It should be noted of course that new lots of yeast were used for each of the experiments. Since several trials of both Experiments I and II were made over a period of several weeks with different lots of yeast, it is reasonable to suppose that the variations are to be found in the peroxide rather than in the yeast. Results in Table I and Fig. 2.

## DISCUSSION OF RESULTS.

Our results, which can best be studied from the curves, demonstrate primarily the great toxicity of hydrogen peroxide for yeast<sup>1</sup>. Although only 3 c.c. of a 30 per cent. solution of peroxide were added during the first two hours, beginning at the fourth hour of the mash, yet there was marked inhibition of each of the important chemical changes occurring in the mash. The yeast growth was almost entirely stopped, the fermentation as measured by total solids and total sugars was greatly retarded, and even the inversion of sucrose by invertase stopped proportionately. During the second addition of 47 c.c. of peroxide over a period of two hours, there was practically entire cessation of all of these changes. However, the final total changes in Experiments I and II were quite different. In the case of Experiment I no recovery had appeared at the end of 26 hours, while in Experiment II almost complete recovery had taken place. We are at a loss to explain this difference. It is probable, however, that the explanation lies in the fact that the catalase of the yeast in Experiment I did not remove the peroxide as rapidly as in the second case. Such a condition would exist if the yeast were especially low in catalase, or if the peroxide contained an impurity inhibitory to catalase action. Chemical analysis of the peroxide showed for the first lot 0.23 gm. of solids per litre, and for the second 1.2 gms. per litre. This residue was largely made up of phosphates. Since several runs were made with each lot of peroxide, and since the yeast used in each run was from a different batch, it is not reasonable to assume a difference in the yeast or in the catalase content of the yeast. It is unfortunate that all of the first lot of the peroxide had been used before the difference was noted, since otherwise further chemical analysis of the ash might have answered this question.

Our experiments, showing the effect of lack of aeration on the rate of yeast growth and fermentation in a molasses mash, have been carried out in commercial laboratories, but so far as we know, have not been reported in the literature, except as noted in the introduction to this paper. The results, Mashers C', Table I, show a decided slowing of yeast multiplication and a much lower total final crop of yeast with practically no increase after the eighth hour. The rates of fermentation and inversion were affected only slightly, since they were complete in eight hours. It is worthy of note that with no aeration there was always a

<sup>1</sup> H. V. Euler and R. Nilsson (*Chem. Zelle u. Gewebe*, XII, 238 (1925) have very recently shown that hydrogen peroxide inhibits fermentation of glucose.

greater total loss of solids than under the other conditions we tried. We believe that this indicates that with no aeration the sugar is fermented more nearly to the theoretical fermentation equation than with aeration. Under aerobic conditions a small portion of the sugar is oxidised into non-volatile organic acids which partially accounts for the decided lowering of  $pH$  as the mash proceeds. In a number of the mashes, the data for which are not given here, we allowed the  $pH$  to change without added bicarbonate. In every case the  $pH$  fell from 7.0 to as low as 2.0 by the eighth hour, thus confirming the findings of H. Luers(4).

It was suggested to us that this very considerable souring of the mash might not be a normal process and that it might come about through contamination of the yeast with lactobacilli. We believe that a contamination sufficient to have produced enough lactic acid to cause such a lowering of  $pH$  would have been observed under the microscope. However, to prove the point, we performed a similar experiment, and used a pure culture of yeast, prepared for us by Prof. C. B. Morrey, of the Department of Bacteriology. The air was washed with bichloride of mercury and concentrated sulphuric acid before passing through the mash. The resultant  $pH$  was 2.5 in nine hours. The probable explanation of the high acidity lies in the fact that the yeast is utilising practically all of the ammonia from the ammonium sulphate and phosphate in the mash, resulting in the liberation of a small amount of free sulphuric and phosphoric acids. About 80 per cent. of the ammonia originally introduced is converted into yeast protein. We believe from these results that this great fall in  $pH$  is a normal process for the type of mash used.

The temporary toxicity of hydrogen peroxide for yeast and its enzymes is analogous, we believe, to the action of hydrocyanic acid. This acid also inhibits yeast growth and enzyme action as long as it is present, but as soon as the acid is removed by artificial means, growth and enzyme action are resumed. In the case of the peroxide, however, the removal of the toxic substance is brought about naturally by the catalase present.

#### SUMMARY.

1. Hydrogen peroxide, when added in the form of superoxol to an aerated yeast molasses mash, is very toxic to the yeast, greatly inhibiting or entirely stopping yeast growth, fermentation and inversion.

2. The yeast yield without aeration in a mash of this kind is greatly diminished, while the other changes are only slightly inhibited, the total loss of solids being appreciably greater than with aeration.

3. If the *pH* change in such an aerated mash is allowed to progress normally, a marked fall from 7.0 to 2.0 is observed, apparently due to the formation of organic acids, and to removal of ammonia from the ammonium salts.

## BIBLIOGRAPHY.

- (1) ALLEN, P. W. (1926). *Industrial Fermentations*, 311.
- (2) BALLS, A. K. and BROWN, J. B. (1925). *J.B.C.* LXII, 823.
- (3) BALLS, A. K. and BROWN, J. B. (1925). *J.B.C.* LXII, 789.
- (4) LUERS, H. (1914). *Z. ges. Brauw.* XXXVII, 79.

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## THE EFFECT OF HEXYL RESORCINOL ON YEAST GROWTH AND FERMENTATION

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IN connection with some studies on yeast metabolism we were interested in determining the toxicity of the new antiseptic, hexyl resorcinol, to ordinary baker's yeast, *Saccharomyces cerevisiae*. This new synthetic is noted both for its bactericidal action, having a phenol coefficient of 45-55, and for its proportionately low toxicity to animal organisms(2,3). For example, a rabbit can tolerate an oral dose of 1 gm. per kg. without harmful effect. There is no indication that it is more toxic to man. In practice it is possible by feeding an adult a gram of the substance in olive oil three times a day to produce actual bactericidal urine in the majority of cases. Our experiments demonstrate that hexyl resorcinol is as toxic to yeast as to bacteria, and further that concentrations of the antiseptic which entirely inhibit yeast growth also completely inhibit alcoholic fermentation.

### EXPERIMENTAL PART.

Since we desired to follow both yeast growth and the chemical changes occurring in the mash, our experiments were arranged so as to facilitate making these determinations. The mash, four litres in volume, contained as follows:

Sucrose ...	...	...	...	...	...	96 gm.
Molasses ...	...	...	...	...	...	64 "
Diammonium phosphate ...	...	...	...	...	...	5 "
Ammonium sulphate ...	...	...	...	...	...	6.4 "
Yeast ...	...	...	...	...	...	11 "

The molasses was dissolved in 500 c.c. of water and centrifugalised to remove sediment. The sucrose was added to this solution and heated just to boiling. The ammonium salts were dissolved and boiled separately. At the beginning of the experiment these two practically sterile solutions were placed in a five litre specimen jar, the yeast in suspension in 50 c.c. of tenth normal sulphuric acid added, and the mixture diluted with

boiled tap water to exactly 4 litres. The fermentation was carried out under conditions favourable for rapid yeast multiplication, *i.e.* under heavy aeration, the air being introduced through a perforated block tin pipe in the bottom of the jar. The air had been previously passed through two wash bottles to saturate it with moisture at the temperature of the experiment, thus avoiding evaporation and change in composition of the mash from its source. The entire apparatus was kept in a thermostat at 28–28.5° C. When the mash had been thoroughly mixed by aeration for two minutes, two portions of 1 litre each were removed by siphon into two other aerating vessels. To these were added dropwise the required amounts of hexyl resorcinol<sup>1</sup> in 10 per cent. alcoholic solution. The rate of aeration was made as nearly equal as possible in the three vessels.

From time to time the three mashes were examined microscopically and analysed for total solids, total sugar, reducing sugar, and yeast crop. The methods used in these determinations are described in a previous paper<sup>(1)</sup>. It is sufficient to state here that the first three components were determined in the centrifugalised beer, and that the yeast crops were estimated by filtering the mash through Gooch crucibles followed by two washings each with water and alcohol and drying for several hours at 107° C. The yeast crops therefore represent the weight of dry yeast substance. No cell counts were made.

In addition to the precautions for sterility described above, all of the containers were sterilised and the mashes kept covered during the experiment. The only sources of contamination would therefore come from the washed air or from the yeast itself. We employed ordinary bakers' yeast, supplied us by the Fleishmann Co. in pound lots. No contamination gross enough to appear under the microscope was found in any of our experiments. The results in our controls are practically identical with those we obtained in other experiments in which we used a pure culture of yeast and sterile air for aeration. On the other hand, the presence of slight infections which might result from the yeast used or from the air would in no way affect our conclusions arrived at in these experiments.

The analytical data and the results of these experiments are shown in Table I, the first series of data comparing the changes in a control and in mashes with 0.1 and 0.3 gm. of hexyl resorcinol per litre, and the second showing the effect of 0.01 and 0.005 gm. of the antiseptic per litre.

<sup>1</sup> Purchased from Sharp and Dohme, Baltimore, Md.

Table I.

*(All data in gms. per litre.)*

Hour of Analysis	<i>Experiment 1</i>			<i>Experiment 2</i>		
	Control	0.3 H.R.	0.1 H.R.	Control	0.01 H.R.	0.005 H.R.
	Total solids					
0	28.75	28.75	28.75	27.76	27.76	27.76
3	27.02	28.05	29.10	25.16	26.18	25.84
6	14.18	28.35	28.75	14.88	16.33	15.78
8	8.53	28.60	28.49	7.79	9.25	9.38
26	6.65	—	28.53	—	—	—
	Total sugar					
0	27.50	27.50	27.50	27.30	27.30	27.30
3	25.91	27.40	27.66	24.72	25.30	24.95
6	10.96	27.50	27.40	13.24	14.14	13.56
8	2.09	27.66	27.59	2.12	3.09	3.22
26	00.00	27.40	27.50	—	—	—
	Reducing sugar					
0	2.32	2.32	2.32	2.93	2.93	2.93
3	6.00	6.72	5.29	17.63	19.72	19.84
6	2.50	10.97	8.59	—	11.71	11.64
8	1.79	12.82	10.17	1.57	2.35	2.73
26	0.00	21.50	20.80	—	—	—
	Yeast crop					
0	0.91	0.91	0.91	0.86	0.86	0.86
3	1.86	0.74	0.90	1.91	1.67	1.75
6	4.56	0.58	0.96	4.61	3.90	4.15
8	5.62	0.77	0.90	6.37	5.28	5.80
26	7.19	0.62	0.84	—	—	—

## DISCUSSION OF RESULTS.

Concentrations of 0.3 and 0.1 gm. of hexyl resorcinol per litre completely inhibited yeast growth and fermentation as measured by loss of total solids and sugar. The higher concentration actually caused a slight loss in yeast weight while microscopically the cells appeared shrunken with no bud formation. It is noteworthy, however, that with each of these concentrations sugar inversion proceeded rapidly with little or no apparent inhibition of invertase action. Concentrations of 0.01 and 0.005 gm. of hexyl resorcinol per litre inhibit only slightly all of the changes, the degree of inhibition being roughly proportional to the amount of antiseptic present. Qualitative tests with fermentation tubes indicated that the sharp break in inhibition of fermentation occurred at a concentration of 0.025 gm. per litre.

From these results one may conclude that the antiseptic action of hexyl resorcinol to yeast is of the same order as that to bacteria, practically complete in 1-10,000. The fact that this antiseptic completely inhibits zymase action (intracellular enzyme) and is without effect on invertase (extracellular action) is of theoretical interest.

#### SUMMARY.

1. Concentrations of 0.3 and 0.1 gm. of hexyl resorcinol per litre completely inhibit yeast growth and fermentation. Concentrations of 0.01 and 0.005 gm. only slightly inhibit.

2. Invertase action is not affected in the presence of 0.3 gm. of hexyl resorcinol per litre of mash.

#### BIBLIOGRAPHY.

- (1) BALLS, A. K. and BROWN, J. B. (1925). *J.B.C.* LXII, 789.
- (2) LEONARD, V. (1924). *J.A.M.A.* LXXXIII, 2005, (1924) *J. Urol.* XII, 585.
- (3) LEONARD, V. and WOOD, A. (1925). *J.A.M.A.* LXXXV, 1855; (1926) *J.A.M.A.* LXXXVI, 668.

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## EARLY MANIFESTATIONS OF POTATO BLIGHT (*PHYTOPHTHORA INFESTANS* DE BARY)

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(With Plate XXVI.)

THE question of the origin of Potato Blight each year is of interest and the following observations seem of value as a corollary to the recent paper of Salmon (1).

On February 17th, 1927, a visit was paid to an experiment on rapid indoor infection of potatoes by Wart Disease for testing purposes at East Craigs carried out by Mr Anderson who said he was troubled by the wilting of potato shoots. The tubers were in a large, airy, well lighted laboratory at room temperature under conditions that, in the winter time especially seemed to indicate freedom from outdoor infection. The potatoes had been well washed, placed on sphagnum moss in saucers and watered at intervals: the whole experiment being carried on indoors. Among the tubers being tested for Wart Disease, several shoots were gathered, about eight in all, that appeared to be suffering from *Phytophthora infestans*, but were not producing spores. In all these shoots a large non-septate mycelium was found. One shoot was about 2 inches long, about  $\frac{1}{3}$  inch thick and very sturdy and was infected from the base upwards for about  $1\frac{1}{4}$  inches with a strong-growing non-septate mycelium. (A similar shoot is shown in Fig. 1.) One side of the shoot was flattened and browned in a typical *P. infestans* manner. From this area of the shoot, sections were taken and the mycelium was observed to start intercellularly. It formed the large lobular hyphae typical of *P. infestans* in the space where three host cells meet. In the more infected areas, the mycelium was intracellular. Small fragments of this tissue were placed in bog water (February 17th) and produced typical *Phytophthora* conidiophores (February 28th). Other shoots were affected towards the apex only, as if they were suffering from a secondary infection. Two or three diseased shoots were put in water in a damp chamber and in 24 hours the first had produced profuse *Phytophthora infestans* conidiophores (18th February). The others followed later with conidiophores.



Fig. 1. Tuber showing healthy shoots and a diseased shoot



Fig. 2. Tuber showing diseased shoot.



On 25th February, a second visit was paid to Mr Anderson's laboratory. In three cases diseased shoots were producing the characteristic fructifications of *Phytophthora infestans*. Many infections were present also that were probably secondary. An especially common place of attack was the small buds in the leaf axils and also the leaves of the young shoots. A noticeable feature was the number of sprouts becoming infected on the under side of the potato, the side next to the sphagnum. These shoots did not progress far but soon rotted away.

It is unlikely in a laboratory during the winter that infection could come in from without. A possible source of infection is from conidio-phores arising on the cut surface of tubers dealt with during the experiments in the laboratory. Although cases of slight infection with Blight have been seen, no conidia on the cut surface, nor extensive infection have been noticed; and all the tubers have been under close observation, both by Mr Anderson and by Miss Simpson.

The evidence points to initially infected shoots, proceeding from infected tubers, producing conidia and these conidia bringing about secondary infection on the other shoots that showed indication of being attacked at the apex first.

Observations would appear to support the theory of initial infection of potato shoots from the diseased tuber by the organism of late Blight *Phytophthora infestans* De Bary.

#### SUMMARY.

Observations during February, 1927, are recorded on nominally healthy potato tubers sprouted indoors in the laboratory of the Seed Testing Station, East Craigs, producing short diseased shoots showing probable primary infection of *Phytophthora infestans*. At the end of the month probable cases of secondary infection occurred.

#### LITERATURE.

- (1) SALMON, E. S. and WARE, W. M. (1926). *Ann. App. Biol.* xii, No. 2, p. 289.

#### • EXPLANATION OF PLATE XXVI.

Fig. 1. Sprouted potato tuber showing one shoot infected with *Phytophthora infestans*, infected from the base upwards. Probable primary infection from small original infection in tuber.

Fig. 2. Sprouted potato tuber showing infection of *Phytophthora infestans* in shoots; apex of shoot infected, probable secondary infection from infected shoots such as shown in Fig. 1. (At a late period shoots such as shown in Fig. 2 were fairly common.)

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# THE INSECT AND OTHER INVERTEBRATE FAUNA OF ARABLE LAND AT ROTHAMSTED. PART II

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(With 3 Text-figures.)

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## 1. INTRODUCTION.

THE investigation which is described in the following paper was carried out between October 1923 and October 1924 and between April and November 1926. Its object was to ascertain the effects of dung and of various artificial manures with and without the addition of dung, on the soil fauna of arable land. A further object was to discover whether there was any noteworthy difference in the species present and their relative numbers and distribution in depth under these conditions as compared with their occurrence in the neighbouring field examined previously<sup>(6)</sup>. The later part of this investigation, that carried out between April and November 1926, was done with a view to supplementing the information obtained earlier, and the results of the two periods are considered together. There had been no change in the manurial treatment of the plots in the interval, the only change being that in 1926 the mangolds were grown on the ridge, whereas previously they had been grown on the flat.

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I am greatly indebted to Dr Imms for many helpful suggestions during the course of this investigation. I am also indebted to Miss K. Warington for information regarding the weeds; to Mr H. J. Page for the chemical analyses; to Dr B. A. Keen for the mechanical analyses; and for assistance in the identification of species of Insecta and "Myriapoda" to Messrs S. G. Brade-Birks, J. M. Brown, H. W. Ellis and G. T. Lyle.

## 2. DESCRIPTION OF AREA EXAMINED.

The field in which the plots examined in the course of this investigation are situated is the Barn Field belonging to the Rothamsted Experimental Station, Harpenden. The soil, as in the case of the field previously examined here, is clay with flints overlying chalk. The Barn Field is roughly rectangular in shape with the long sides running S. by W. to N. by E., and, at its lowest part, the elevation is just above the 400 feet contour line.

The field is divided longitudinally into eight strips running the whole length of the field, each strip receiving one manure throughout its length. The strips are then subdivided into plots by cross-dressings of other manures, there being altogether 40 experimental plots.

Mangolds have been grown on this field since 1876 and in plots used in this investigation, the manurial treatment they receive and their yield of mangolds during 1924 are:

No. O 8, Unmanured (control): 2.14 tons.

No. A 8, Ammonium salts only: 10.18 tons.

No. O 5, Superphosphate only: 3.31 tons.

No. O 1, Dung only: 14.49 tons.

No. O 2, Dung, superphosphate and sulphate of potash: 18.61 tons.

No. A 1, Dung and ammonium salts: 20.75 tons.

The quantities of the different manures applied per acre are: dung 14 tons; ammonium salts 400 lb., consisting of equal parts of sulphate and muriate of ammonia of commerce; superphosphate 3.5 cwt.; and sulphate of potash 500 lb., this having been applied to Plot O 2 only since 1895. The "O" series of plots lies along the northern end of the field, and the "A" series lies across the field near the middle of its length. Each plot is rather over one-seventh of an acre in area. The effect of the different manurial treatments on the crop is very noticeable in their yield of roots and leaves, as well as in the general appearance of the mangolds and weeds.

The field was ploughed during the second week in February 1924.

*Meteorological Conditions.*

No records of meteorological conditions are given in the present paper. Barn Field adjoins the meteorological station of the Rothamsted Experimental Station and the records obtained here and elsewhere on the station are available. As these conditions would be more or less uniform for the different plots examined, they are not considered as part of the causes affecting the distribution of the fauna over the various plots.

## 3. METHOD OF INVESTIGATION.

The samples of soil which were examined during this investigation were taken along one of the long edges of the plots, except on one or two occasions when the ground was snow-covered, samples being then taken well into the plots to avoid risk of missing the plot altogether. Successive samples from a plot were spaced well apart.

The samples were taken with the same apparatus of iron plates and trowel as was described previously<sup>(6)</sup>; in addition a square iron frame was used which helps considerably to hold the plates in position whilst they are being driven into the ground. One side of this frame is removable, fitting into place over studs on the larger portion, this side being taken out to facilitate removal of the soil between the plates, this side of the frame and the smallest plate coming on the same side of the sample. The size of the frame is such that the plates will just fit inside it with a minimum of clearance, and slots are arranged in it to take the excess width of the largest plates, and hold them in position. With this frame in use it is considerably easier to drive the plates into the soil in their correct relative positions and the volume of soil enclosed by them is more likely to be correct, the plates being less liable to be displaced by striking against stones.

As before the samples of soil were each a nine inch cube, each sample being removed in five layers consisting of: I, the soil between the surface and a depth of one inch below the lowest point of the surface; II, the soil between a depth of one inch below the lowest point of the surface and a depth of three inches; III, the soil between three inches and five inches; IV, the soil between five inches and seven inches; V, the soil between seven inches and nine inches.

Each layer was placed in a linen bag and removed to the laboratory, where the soil was placed in the washing apparatus described previously (*Bull. Ent. Res.* XIII, 1922). If stirred constantly a bagful of soil could be completely washed in an hour or less, but usually it was more con-

venient to leave it longer, only stirring occasionally. After the washing was completed the residues on the two finer sieves of the apparatus were examined, a small portion at a time, in water in a white basin. The residue in the coarse sieve was also examined but did not require such detailed examination as that in the finer sieves, owing to the large size of any insects, etc., retained by it. Many forms floated on the surface of the water in the basin and the remainder could be readily seen through the clear water. Altogether 36 soil samples were taken. Seven samples of soil were examined from each of the plots without dung (O 8, A 8, O 5) and five samples from each of the plots with dung (O 1, O 2, A 1). The samples from the undunged plots were taken from October 1923 to March 1924, and those from the dunged plots from March to October 1924.

#### 4. SOIL ANALYSES.

The following table gives the mechanical analyses of the soil of the plots:

Plot numbers and depth of samples	Water in air dry soil %	Loss in solu- tion %	Coarse sand %	Fine sand %	Silt %	Fine silt I %	Fine silt II %	Clay %	Loss on ig- nition %
O 8 surface to 5"	1.9	0.9	8.0	18.4	27.4	5.6	2.6	23.6	7.2
5" to 9"	3.3	0.9	6.2	13.9	20.4	7.0	1.2	36.4	8.3
A 8 surface to 5"	1.9	0.7	6.7	21.9	34.8	10.0	4.6	20.8	5.9
5" to 9"	3.3	0.7	4.2	16.2	24.6	6.8	2.8	33.6	7.4
O 5 surface to 5"	2.0	1.1	8.2	20.7	29.0	11.2	0.8	20.6	6.7
5" to 9"	2.5	1.1	8.9	18.5	23.4	7.6	3.2	27.2	6.6
O 1 surface to 5"	2.6	1.0	7.1	15.8	27.8	7.6	2.0	23.6	9.8
5" to 9"	3.2	0.6	5.7	14.4	23.4	7.2	2.6	32.8	9.1
O 2 surface to 5"	2.3	1.0	7.4	18.1	8.6	27.8	1.6	22.0	9.7
5" to 9"	3.3	0.5	4.3	13.1	19.6	11.4	0.6	35.4	8.6
A 1 surface to 5"	2.5	1.0	8.2	19.4	29.0	9.6	2.2	18.6	9.1
5" to 9"	2.0	0.9	7.2	19.3	30.2	9.2	3.0	17.4	7.9

Chemical analyses have also been obtained of the soil of the plots, but as they do not indicate any very important differences between the various plots which can be correlated with differences in the soil fauna, it is not considered necessary to publish them. These figures are however being kept so that they can be referred to at any time if necessary.

#### 5. OCCURRENCE OF WEEDS.

Weeds are usually fairly abundant on Barn Field, although only a few species are important. Owing to the nature of the crop, cleaning operations can be carried out quite conveniently during most of the year, so that there is never a mass of weeds such as is found on Broadbalk.



*Cirsium arvense* is the only important perennial; of the plots under consideration it is most plentiful on those not receiving dung. *Capsella bursa-pastoris* and *Veronica hederifolia* are abundant, and *Stellaria media* is also important.

## 6. SOIL FAUNA OF EXPERIMENTAL PLOTS SUBJECTED TO VARIED KINDS OF MANURING.

In the following lists the worms have been divided into two groups, those belonging to the sub-order Terricolae of the order Oligochaeta, which includes the true earthworms, *Lumbricus*, etc., forming one group as Oligochaeta (Terricolae), and all other worms, probably principally belonging to the family Enchytraeidae of the Oligochaeta, and to the Nematoda, forming the second group as Oligochaeta (Limicolae), etc.

The numbers following the names have the following meaning: the first numbers give the months during which the species was met with; the first numbers within the brackets give, above, the total number found, and below, in Roman numerals, the levels in which they were found. The second numbers within the brackets give, above, the greatest number found at any one level, and below, in Roman numerals, the level at which they were found.

Thus—*Onychiurus fimetarius* (L.) 3, 4, 5, 6, 10  $\left( \begin{smallmatrix} 26 & 11 \\ \text{I-V} & \text{II} \end{smallmatrix} \right)$  indicates that this species was found in the months March to June and October; 26 were found altogether, occurring in each of the five layers, from the surface to a depth of nine inches, and 11 of these were found in the second layer, between a depth of one inch and three inches.

The species of Insects and other Invertebrates present in the plots were as follows:

### Plot No. O 8. Unmanured (Control).

Samples taken in months 10, 11, 12, 1, 2, 4, 6, 10.

#### INSECTA.

**Collembola.** *Onychiurus armatus* (Tullb.) 11, 12, 1, 2, 4, 6, 10  $\left( \begin{smallmatrix} 36 & 19 \\ \text{I-III} & \text{II} \end{smallmatrix} \right)$ ; *Achorutes purpureus* Lubbock 11, 12  $\left( \begin{smallmatrix} 6 & 4 \\ \text{II, V} & \text{V} \end{smallmatrix} \right)$ ; *Isotoma griseus* Schöff. 10  $\left( \begin{smallmatrix} 1 \\ \text{I} \end{smallmatrix} \right)$ ; *I. minor* Schöff. 10  $\left( \begin{smallmatrix} 4 \\ \text{V} \end{smallmatrix} \right)$ ; *Orchesella villosa* (Geoff.) 6  $\left( \begin{smallmatrix} 1 \\ \text{IV} \end{smallmatrix} \right)$ ; *Lepidocyrtus cyaneus* Tullb. 6  $\left( \begin{smallmatrix} 1 \\ \text{III} \end{smallmatrix} \right)$ .

**Pseudoscorpiones.** Sp. 10  $\left( \begin{smallmatrix} 1 \\ \text{II} \end{smallmatrix} \right)$ .

**Thysanoptera.** Sp. 4, 10  $\left( \begin{smallmatrix} 2 \\ \text{III} \end{smallmatrix} \right)$ .

**Hemiptera.** *Anthocoris nemoralis* F. 10  $\left(\frac{1}{II}\right)$ .

**Coleoptera.** *Helophorus nubilus* F. 11  $\left(\frac{1}{II}\right)$ ; *Atheta (Meotica) exilis* Er. 1  $\left(\frac{1}{I}\right)$ ; *Homalota* sp. 4  $\left(\frac{1}{II}\right)$ ; *Aleochara* sp. 4  $\left(\frac{1}{I}\right)$ ; *Oxytelus tetracaratus* Block. 6  $\left(\frac{2}{IV-V}\right)$ ; *Scopaeus sulcicollis* Steph. 12  $\left(\frac{1}{IV}\right)$ ; *Atomaria linearis* Steph. 12, 2, 10  $\left(\frac{4}{III-V}, \frac{2}{III}\right)$ ; *Anthicus antherinus* L. 2  $\left(\frac{1}{II}\right)$ ; *Ceuthorrhynchus quadridens* Panz. 4  $\left(\frac{1}{IV}\right)$ . Larvae: Sp. 10  $\left(\frac{1}{III}\right)$ ; CARBIDAE 10, 1  $\left(\frac{3}{I-III}\right)$ ; NERBRIA sp. 1, 2  $\left(\frac{2}{I}\right)$ ; STAPHYLINIDAE 10  $\left(\frac{1}{I}\right)$ ; ELATERIDAE, *Agriotes* sp. 10  $\left(\frac{1}{III}\right)$ .

**Diptera.** CECIDOMYIDAE. Larvae in grass stem sp. 1  $\left(\frac{2}{I}\right)$ , larvae sp. 4  $\left(\frac{1}{IV}\right)$ . SCATO-PSIDAE. Sp. (larvae) 6  $\left(\frac{1}{III}\right)$ . EMPIDAE. Sp. 6  $\left(\frac{1}{IV}\right)$ . PHORIDAE. *Phora* sp. 6  $\left(\frac{1}{IV}\right)$ ; sp. 10  $\left(\frac{1}{II}\right)$ . BORBORIDAE. *Limosina* sp. 6  $\left(\frac{1}{II}\right)$ . ANTHOMYIDAE. *Leptohylemyia coarctata* Fall. (eggs) 10, 12, 1  $\left(\frac{5}{I}, \frac{3}{II}, \frac{1}{I}\right)$ .

**Hymenoptera.** FORMICIDAE. Sp. 6  $\left(\frac{1}{I}\right)$ . BRACONIDAE. *Blacus* sp. 10  $\left(\frac{1}{II}\right)$ .

**DIPLOPODA.** Non det. 10, 11, 12  $\left(\frac{9}{I-V}, \frac{3}{III}\right)$ . POLYDESMIDAE. Sp. 4, 10  $\left(\frac{5}{I-III}, \frac{2}{II}, \frac{1}{III}\right)$ . BLANIULIDAE. (Probably mainly *Blaniulus guttulatus* (Bosc.), 10, 2, 4, 6  $\left(\frac{13}{I-V}, \frac{4}{III}\right)$ .

**SYMPHYLA.** Sp. 10  $\left(\frac{1}{V}\right)$ .

**ARACHNIDA. Araneida.** Sp. 11, 12, 6  $\left(\frac{3}{I}\right)$ .

**Acarina.** Sp. 10, 11, 12, 6  $\left(\frac{4}{I-V}\right)$ .

**OLIGOCHAETA (Terricolae).** 10, 4  $\left(\frac{3}{I, IV, V}\right)$ .

**OLIGOCHAETA (Limicolae).** 4, 6, 10  $\left(\frac{29}{I-V}, \frac{12}{II}\right)$ .

**ISOPODA.** 11  $\left(\frac{2}{III, IV}\right)$ .

### Plot No. A 8. (Ammonium Salts.)

Samples taken in months 11, 12, 1, 2, 3, 4, 5, 10.

### INSECTA.

**Collembola.** *Anurida granaria* (Nic.) Tullb. 12, 1  $\left(\frac{2}{II, III}\right)$ ; *Onychiurus armatus* (Tullb.) 11, 12, 1, 2, 4, 10  $\left(\frac{75}{I-V}, \frac{45}{II}\right)$ ; *O. fmetarius* (L.) 2  $\left(\frac{1}{IV}\right)$ ; *O. ambulans* (L.) 12  $\left(\frac{2}{IV}\right)$ ; *Achorutes purpureascens* Lubb. 11, 1  $\left(\frac{2}{V}\right)$ ; *Isotomurus palustris* (Mull.) 11  $\left(\frac{1}{I}\right)$ ; *Entomobrya nivialis* (L.) 1  $\left(\frac{1}{III}\right)$ .

**Orthoptera.** *Ectobius lapponicus* L. 11  $\left(\frac{1}{V}\right)$ ; *Forficula auricularia* L. 11, 3  $\left(\frac{2}{II, III}\right)$ , eggs 3  $\left(\frac{36}{III}\right)$ .

**Thysanoptera.** Sp. 11, 10  $\left(\frac{3}{I}\right)$ .

**Hemiptera.** Sp. (larva) 5  $\left(\frac{1}{III}\right)$ . **APHIDIDAE** Sp. 10  $\left(\frac{2}{I}\right)$ .

**Coleoptera.** *Xantholinus punctulatus* Payk. 5  $\left(\frac{1}{III}\right)$ ; *Oxytelus tetracarinatus* Block 4  $\left(\frac{1}{III}\right)$ ; *O. inustus* Grav. 5  $\left(\frac{1}{IV}\right)$ ; *O. nitidulus* Grav. 10  $\left(\frac{1}{III}\right)$ ; *Stenus subaeneus* Er. 1  $\left(\frac{1}{I}\right)$ ; *Trichopteryx* sp. 4  $\left(\frac{1}{III}\right)$ ; *Euplectus piceus* Mots. 5  $\left(\frac{1}{IV}\right)$ ; *Atomaria linearis* Steph. 10  $\left(\frac{1}{IV}\right)$ . Larvae: **CARABIDAE** 11  $\left(\frac{2}{I, II}\right)$ ; **STAPHYLINIDAE** 4, 10  $\left(\frac{2}{I, II}\right)$ .

**Diptera.** **CECIDOMYIDAE.** Sp. (larva) 4  $\left(\frac{1}{IV}\right)$ , (pupa) 4  $\left(\frac{1}{III}\right)$ . **MYCETOPHYLIDAE.** Sp. 3  $\left(\frac{1}{III}\right)$ . **CHIRONOMIDAE.** Sp. (larvae) 4, 5  $\left(\frac{3}{I, II, I}\right)$ , 2  $\left(\frac{2}{I, II, I}\right)$ . **TIPULIDAE.** *Trichocera* sp. (larvae) 2, 4, 5  $\left(\frac{7}{II-IV}, \frac{3}{IV}\right)$ . **BIBIONIDAE.** *Dilophus* (larvae) 2  $\left(\frac{6}{I-II}, \frac{5}{II}\right)$ . **ANTHOMYIDAE.** Sp. (pupa) 10  $\left(\frac{1}{I}\right)$ ; *Leptohylemyia coarctata* Fall. (eggs) 11, 12, 1, 10  $\left(\frac{27}{I-III}, \frac{19}{I}\right)$ .

**Hymenoptera.** **BRACONIDAE.** Sp. 5  $\left(\frac{1}{I}\right)$ .

**DIPLOPODA.** Non det. 11, 12  $\left(\frac{2}{II, III}\right)$ . *Ophiodesmus albonatus* (Latzel) 3  $\left(\frac{1}{V}\right)$ . **BLANIULIDAE.** Sp. 4  $\left(\frac{1}{II}\right)$ .

**ARACHNIDA.** **Araneida.** Sp. 4  $\left(\frac{1}{III}\right)$ .

**Acarina.** 11, 12, 4  $\left(\frac{19}{II, III, III}\right)$ .

**OLIGOCHAETA (Terricolae).** 5, 10  $\left(\frac{2}{I, II}\right)$ .

**OLIGOCHAETA (Limicolae).** 11, 4, 5, 10  $\left(\frac{44}{I-V}, \frac{19}{II}\right)$

Plot No. O 5. Superphosphate only.

Samples taken in months 11, 12, 1, 2, 3, 4, 6, 11.

#### INSECTA.

**Collembola.** *Anurida granaria* (Nic.) Tullb. 11, 1  $\left(\frac{2}{III, IV}\right)$ . *Onychiurus armatus* (Tullb.) 11, 12, 1, 2, 5, 6, 11  $\left(\frac{141}{I-V}, \frac{59}{II, III}\right)$ ; *O. ambulans* (L.) 11  $\left(\frac{5}{IV}\right)$ ; *Achorutes purpureascens* Lubb. 11, 12  $\left(\frac{9}{I, III, IV}, \frac{4}{III, IV}\right)$ ; *Entomobrya muscorum* (Tullb.) 1  $\left(\frac{1}{II}\right)$ ; *E. marginata* Tullb. 6  $\left(\frac{1}{III}\right)$ ; *Isotomurus palustris* (Mull.) 12  $\left(\frac{1}{I}\right)$ .

**Thysanoptera.** Sp. 5  $\left(\frac{1}{I}\right)$ .

**Hemiptera.** **APHIDIDAE.** Sp. 6  $\left(\frac{1}{I}\right)$ .

**Lepidoptera.** **NOCTUIDAE.** Sp. (Larva) 11  $\left(\frac{1}{I}\right)$ .

**Coleoptera.** *Helophorus Mulsanti* Rye 6  $\left(\frac{1}{III}\right)$ ; *Homalota* sp. 5, 6  $\left(\frac{3}{I, IV, V}\right)$ ; *Oxytelus nitidulus* Grav. 11  $\left(\frac{1}{III}\right)$ ; *Atomaria linearis* Steph. 11  $\left(\frac{2}{IV, V}\right)$ ; *Haltica* sp. 11  $\left(\frac{1}{I}\right)$ . Larvae: CARABIDAE 11, 1  $\left(\frac{2}{II, III}\right)$ ; STAPHYLINIDAE 11  $\left(\frac{1}{I}\right)$ ; sp. 11  $\left(\frac{1}{II}\right)$ .

**Diptera.** CECIDOMYIDAE. (Larva) 1  $\left(\frac{1}{I}\right)$ . TIPULIDAE. Sp. (larva) 11  $\left(\frac{1}{I}\right)$ ; *Trichocera* sp. (larva) 11  $\left(\frac{2}{II}\right)$ . SCATOPIIDAE. Sp. (larva) 11  $\left(\frac{1}{III}\right)$ . ANTHOMYIDAE. *Leptohylemyia coarctata* Fall. (eggs) 1, 11  $\left(\frac{3}{I, II, II}\right)$ . MUSCIDAE. Sp. (parasitised puparium) 11  $\left(\frac{1}{I}\right)$ .

**Hymenoptera.** FORMICIDAE. *Donisthorpea flava* F. 11  $\left(\frac{1}{II}\right)$ . BRACONTIDAE. *Alysia rufidens* Nees. 11  $\left(\frac{1}{I}\right)$  (reared from Muscid puparium).

**CHILOPODA.** *Geophilus longicornis* Leach 5  $\left(\frac{1}{III}\right)$ .

**DIPLOPODA.** Non det. 11  $\left(\frac{1}{V}\right)$ . *Ophiodesmus albonatus* (Latzel). 11  $\left(\frac{1}{IV}\right)$ ; sp. 11  $\left(\frac{4}{I, III}, \frac{3}{I}\right)$ . BLANIULIDAE. Sp. 6  $\left(\frac{2}{II, III}\right)$ .

**ARACHNIDA.** Araneida. Sp. 11  $\left(\frac{1}{I}\right)$ .

Acarina. Sp. 11  $\left(\frac{1}{III}\right)$ .

**OLIGOCHAETA** (Limicolae). 11, 1, 2, 5, 6, 11  $\left(\frac{27}{I-V}, \frac{12}{III}\right)$ .

### Plot No. O 1. Dung only.

Samples taken during months 3, 4, 5, 6, 10.

### INSECTA.

**Collembola.** Non det. 6  $\left(\frac{1}{V}\right)$ . *Anurida granaria* (Nic.) Tullb. 4, 5, 6, 10  $\left(\frac{50}{I-V}, \frac{18}{III}\right)$ ; *Onychiurus finetarius* (L.) 3, 4, 5, 6, 10  $\left(\frac{26}{I-V}, \frac{11}{II}\right)$ ; *O. ambulans* (L.) 6  $\left(\frac{2}{IV, V}\right)$ ; *Achorutes purpureascens* Lubbo. 3, 4, 5, 6  $\left(\frac{11}{I-IV}, \frac{5}{III}\right)$ ; *Folsomia finetaria* (L.) 5  $\left(\frac{2}{II, III}\right)$ ; *Isotoma* sp. 4, 5  $\left(\frac{3}{II, III}, \frac{2}{II}\right)$ ; *Isotomurus palustris* (Mull.) 6, 10  $\left(\frac{6}{I}\right)$ ; *Heteromurus nitidus* (Templ.) 10  $\left(\frac{1}{I}\right)$ .

**Orthoptera.** *Forficula auricularia* L. (nymph) 10  $\left(\frac{1}{III}\right)$ .

**Thysanoptera.** Sp. 5  $\left(\frac{1}{II}\right)$ .

**Coleoptera.** CARABIDAE. Larvae 3, 4, 5  $\left(\frac{9}{I, III, V}, \frac{4}{III}\right)$ . STAPHYLINIDAE. *Atheta* (*Meotica*) *exilis* Er. 4, 10  $\left(\frac{5}{I, II}, \frac{3}{II}\right)$ ; *Tachyporus hymnorum* F. 5  $\left(\frac{1}{I}\right)$ . Larvae 4, 5  $\left(\frac{2}{I, V}\right)$ . CRYPTOPHAGIDAE. *Atomaria linearis* Steph. 4, 5  $\left(\frac{3}{I, V}, \frac{2}{I}\right)$ . ELATERIDAE. *Athous* sp. (larvae) 4, 5, 6, 10  $\left(\frac{6}{II-V}, \frac{2}{IV, V}\right)$ . Larva non det. 10  $\left(\frac{1}{I}\right)$ .

**Diptera.** CECIDOMYIDAE. Larvae 5  $\left(\frac{2}{II, III}\right)$ . CHIRONOMIDAE. Larvae 4, 5  $\left(\frac{5}{II, III}, \frac{4}{II}\right)$ . TIPULIDAE. *Trichocera* sp. (larvae) 5, 10  $\left(\frac{5}{I-III}, \frac{2}{II, III}\right)$ . EMPIDAE. Larva 5  $\left(\frac{1}{I}\right)$ . PHORIDAE. Larvae 3  $\left(\frac{2}{III}\right)$ . ANTHOMYIDAE. Puparia 3, 4, 5  $\left(\frac{3}{I, II}, \frac{2}{II}\right)$  (one parasitised).

**Hymenoptera.** IOHNEUMONIDAE. *Phygadeuon fumator* Grav. 5  $\left(\frac{1}{II}\right)$  (reared from Anthomyid puparium).

“MYRIAPODA.”

**Chilopoda.** GEOPHILIDAE. *Geophilus longicornis* Leach. 3, 5, 6  $\left(\frac{7}{I-IV}, \frac{5}{II}\right)$ .

**Diplopoda.** POLYDESMIDAE (probably mostly *Brachydesmus superus mosellanus* Verhoeff) 3, 4, 5, 6, 10  $\left(\frac{20}{I-V}, \frac{12}{I}\right)$ ; *Brachydesmus superus mosellanus* Verhoeff. 3, 5, 6, 10  $\left(\frac{15}{I-V}, \frac{5}{II}\right)$ ; *Ophiodesmus albonanus* (Latzel) 6  $\left(\frac{2}{II}\right)$ . IULIDAE. *Cylindroiulus londinensis* var. *caerulescinctus* (Wood) 5, 6  $\left(\frac{8}{I-IV}, \frac{5}{II}\right)$ . BLANIULIDAE. (Probably mostly *Blaniulus guttulatus* (Bosc.) 3, 4, 5, 6, 10  $\left(\frac{43}{I-V}, \frac{14}{III}\right)$ . *Blaniulus guttulatus* (Bosc.) 3, 4, 5, 6  $\left(\frac{38}{I-IV}, \frac{11}{III}\right)$ .  
**Symphyla.** Sp. 5, 6  $\left(\frac{2}{III}\right)$ .

ARACHNIDA.

**Araneida.** Sp. 4  $\left(\frac{1}{I}\right)$ .

**Acarina.** 3, 4, 5, 6, 10  $\left(\frac{14}{I-V}, \frac{5}{II}\right)$ .

**OLIGOCHAETA (Terricolae).** 3, 4, 5, 6, 10  $\left(\frac{62}{I-IV}, \frac{24}{II}\right)$ .

**OLIGOCHAETA (Limicolae).** 3, 4, 5, 6, 10  $\left(\frac{462}{I-V}, \frac{162}{II}\right)$ .

Plot No. O 2. Dung, Superphosphate and Potash.

Samples taken during months 3, 4, 5, 6, 10.

INSECTA.

**Collembola.** *Anuria granaria* (Nic.) Tullb. 3, 4, 5, 6, 10  $\left(\frac{16}{I-V}, \frac{5}{II}\right)$ . *Onychiurus armatus* (Tullb.) 3, 4, 6  $\left(\frac{5}{I, III, IV}, \frac{3}{I}\right)$ ; *O. fimetarius* (L.) 3, 4, 5, 6, 10  $\left(\frac{50}{I-IV}, \frac{24}{II}\right)$ ; *O. ambulans* (L.) 4, 5  $\left(\frac{5}{I, II}, \frac{4}{II}\right)$ ; *Achorutes purpurescens* Lubh. 4, 5, 6  $\left(\frac{15}{I-V}, \frac{5}{I, II}\right)$ ; *Isotomurus palustris* (Mull.) 4  $\left(\frac{2}{II, V}\right)$ ; *Lepidocyrtus lanuginosus* (Gmel.) 5  $\left(\frac{3}{III}\right)$ ; sp. 10  $\left(\frac{1}{III}\right)$ ; *Bourletiella hortensis* (Fitch) 6  $\left(\frac{1}{I}\right)$ .

**Hemiptera.** JASSIDAE. Sp. 10  $\left(\frac{1}{II}\right)$ . ARTHRIDAE. *Geocia* sp. 10  $\left(\frac{3}{II}\right)$ .

**Coleoptera.** CARABIDAE. Larvae 4, 5  $\left(\frac{2}{II, III}\right)$ . HYDROPHYLIDAE. *Helophorus nubilus* F. 4  $\left(\frac{1}{II}\right)$ . STAPHYLINIDAE. *Atheta (Meolva) exilis* Er. 3, 4, 5, 10  $\left(\frac{15}{I-V}, \frac{3}{II}\right)$ ; *Tachysa umbratica* Er. 3  $\left(\frac{1}{I}\right)$ ; *Aleochara tristis* Grav. 5  $\left(\frac{1}{II}\right)$ . CRYPTOPHAGIDAE. *Atomaria linearis* Steph. 4, 5, 6  $\left(\frac{3}{I-II}, \frac{2}{I}\right)$ . ELATERIDAE. *Athous* sp. (larvae) 3, 4, 6  $\left(\frac{3}{IV, V}, \frac{2}{IV}\right)$ . Larvae non det. 10  $\left(\frac{1}{I}\right)$ .

**Diptera.** CECIDOMYIDAE. Larvae 5, 6  $\left(\frac{2}{I, III}\right)$ . MYCETOPHILIDAE. Sp. 5  $\left(\frac{1}{II}\right)$ .

CHIRONOMIDAE. Larvae 5, 6  $\left(\frac{4}{I, II}\right)$ . TIPULIDAE. Larva 3  $\left(\frac{1}{III}\right)$ ; *Trichocera* (larvae) 3, 10  $\left(\frac{2}{I, III}\right)$ . PHORIDAE. Sp. 10  $\left(\frac{1}{I}\right)$ .

### "MYRIAPODA."

**Chilopoda.** GEOPHILIDAE. *Geophilus longicornis* Leach 3, 4, 5, 10  $\left(\frac{7}{II-V}, \frac{2}{II, IV, V}\right)$ .

**Diplopoda.** Non det. 3, 10  $\left(\frac{7}{IV, V}, \frac{5}{IV}\right)$ . POLYDESMIDAE. (Probably mostly *Brachydesmus superus mosellanus* Verhoeff) 3, 4, 5, 6, 10  $\left(\frac{21}{I-V}, \frac{9}{II}\right)$ ; *Brachydesmus superus mosellanus* Verhoeff 3, 5  $\left(\frac{4}{II}\right)$ ; *Ophiodesmus albonanus* (Latzel) 3, 5  $\left(\frac{6}{I}\right)$ . JULIDAE. *Cylindroiulus londinensis* var. *caeruleocinctus* (Wood) 4, 5, 6, 10  $\left(\frac{9}{I-V}, \frac{5}{II}\right)$ . BLANIULIDAE. (Probably mostly *Blaniulus guttulatus* (Bosc.)) 4, 5, 6, 10  $\left(\frac{53}{I-V}, \frac{20}{II}\right)$ ; *Blaniulus guttulatus* (Bosc.) 3, 4, 5, 6, 10  $\left(\frac{35}{II-V}, \frac{24}{III}\right)$ ; *Archiboreoiulus pallidus* (Brade-Birks) 4, 6  $\left(\frac{10}{II-III}, \frac{9}{III}\right)$ .  
**Symphyla.** Sp. 3, 5  $\left(\frac{2}{III, IV}\right)$ .

### ARACHNIDA.

**Araneida.** Sp. 3, 4  $\left(\frac{2}{I, III}\right)$ .

**Acarina.** 3, 4, 5, 10  $\left(\frac{11}{I-IV}, \frac{6}{III}\right)$ .

**OLIGOCHAETA (Terricolae).** 3, 4, 5, 6, 10  $\left(\frac{36}{I, IV}, \frac{13}{I}\right)$ .

**OLIGOCHAETA (Limicolae).** 3, 4, 5, 6, 10  $\left(\frac{255}{I-V}, \frac{120}{III}\right)$ .

### Plot No. A 1. Dung and Ammonium Salts.

Samples taken during months 4, 5, 8, 10.

### INSECTA.

**Collembola.** *Anurida granaria* (Nic.) Tullb. 4, 5, 8, 10  $\left(\frac{55}{I-V}, \frac{15}{IV}\right)$ ; *Onychiurus armatus* (Tullb.) 4, 5, 8, 10  $\left(\frac{32}{I-IV}, \frac{16}{II}\right)$ ; *O. finetarius* (L.) 4, 5, 8, 10  $\left(\frac{29}{II-V}, \frac{15}{III}\right)$ ; *O. ambulans* (L.) 8  $\left(\frac{1}{IV}\right)$ ; *Achorutes purpurescens* Lubb. 5  $\left(\frac{10}{I-V}, \frac{6}{II}\right)$ ; *Folsomia finetaria* (L.) 5  $\left(\frac{1}{I}\right)$ ; *Isotoma griseocens* Schäff. 4  $\left(\frac{1}{II}\right)$ ; *Isotomurus palustris* (Mull.) 8, 10  $\left(\frac{3}{I, III}, \frac{2}{I}\right)$ ; *Heteromurus nitidus* (Temp.) 5  $\left(\frac{2}{III, IV}\right)$ ; *Lepidocyrtus sex-oculatus* (Schott) 8  $\left(\frac{1}{III}\right)$ ; *Bourletiella hortensis* (Fitch) 5  $\left(\frac{2}{I}\right)$ .

**Hemiptera.** HOMOPTERA (larva) 5  $\left(\frac{1}{I}\right)$ .

**Coleoptera.** CARABIDAE. *Amara familiaris* Duft. 10  $\left(\frac{1}{I}\right)$ ; *Pterostichus* (larva) 4  $\left(\frac{1}{III}\right)$ ; larvae 4, 5  $\left(\frac{4}{II, III}, \frac{3}{II}\right)$ . STAPHYLINIDAE. *Oxytelus nitidulus* Grav. 4  $\left(\frac{1}{I}\right)$ ; *O. tetracaratus* Block. 5  $\left(\frac{1}{III}\right)$ ; *Scopaeus sulcicollis* Steph. 8  $\left(\frac{1}{I}\right)$ ; larvae 4, 8  $\left(\frac{11}{I-III}, \frac{7}{III}\right)$ .

CRYPTOPHRAGIDAE. *Atomaria linearis* Steph. 4, 5  $\left(\frac{24}{I-V}, \frac{11}{I}\right)$ . ELATERIDAE. *Athous* sp. 8  $\left(\frac{2}{II, V}\right)$ . Larvae non det. 8, 10  $\left(\frac{18}{I, II}, \frac{11}{II}\right)$ .

**Diptera.** MYCETOPHILIDAE. Larva 4  $\left(\frac{1}{II}\right)$ . CHIRONOMIDAE. Larva 4  $\left(\frac{1}{II}\right)$ . TIPULIDAE. *Trichocera* sp. (larvae) 4, 5, 8  $\left(\frac{91}{II-IV}, \frac{66}{III}\right)$ . PHORIDAE. Larvae 4  $\left(\frac{4}{I, II}\right)$ . ANTHOMYIDAE. Pupa 4  $\left(\frac{1}{II}\right)$ . Non det. pupa 4  $\left(\frac{1}{II}\right)$ .

#### "MYRIAPODA.

**Chilopoda.** LITHOBIDAE. *Monotarsobius dubosqui* (Brolemann) 5  $\left(\frac{1}{II}\right)$ . GEO-  
PHILIDAE. *Geophilus longicornis* Leach 5, 8, 10  $\left(\frac{11}{I-V}, \frac{6}{III}\right)$ .

**Diplopoda.** POLYDESMIDAE. (Probably mainly *Brachydesmus superus mosellanus* Verhoeff) 4, 5, 8, 10  $\left(\frac{25}{I-V}, \frac{8}{I}\right)$ ; *Brachydesmus superus mosellanus* Verhoeff 5  $\left(\frac{3}{IV}\right)$ ; *Ophiodesmus albanus* (Latzel) 10  $\left(\frac{1}{IV}\right)$ . CRASPEDOSOMIDAE. *Polymicrodon polydesmoides* (Leach) 5  $\left(\frac{1}{III}\right)$ . BLANIULIDAE. (Probably mainly *Blaniulus guttularius* (Bosc.)) 4, 5, 8, 10  $\left(\frac{15}{I-V}, \frac{7}{II}\right)$ ; *Blaniulus guttularius* (Bosc.) 5  $\left(\frac{5}{III}, \frac{3}{V}, \frac{3}{V}\right)$ ; *Archiboreoiulus pallidus* (Brade-Birks) 5  $\left(\frac{2}{IV}\right)$ .

**Symphyla.** Sp. 4, 5, 8  $\left(\frac{3}{I, II, IV}\right)$ .

#### ARACHNIDA.

**Araneida.** Sp. 8, 10  $\left(\frac{2}{I}\right)$ .

**Acarina.** 4, 5, 8  $\left(\frac{6}{II-IV}, \frac{4}{II}\right)$ .

**OLIGOCHAETA (Terricolae).** 4, 5, 8, 10  $\left(\frac{40}{I-IV}, \frac{16}{I}\right)$ .

**OLIGOCHAETA (Limicolae).** 4, 5, 8, 10  $\left(\frac{260}{I-V}, \frac{105}{III}\right)$ .

**PULMONATA.** 8, 10  $\left(\frac{2}{I}\right)$ .

### 7. CENSUS OF THE FAUNA OF THE PLOTS EXAMINED.

(a) *Unmanured (Control) Plot (No. O 8).* The total number of Invertebrates in this plot in ten samples was 156, or 1,208,000 per acre. Of these 87, or 673,000 per acre, were Insects. In addition five eggs of *Leptohylemyia coarctata* (39,900 per acre) were found in this plot.

The numbers per acre of the more abundant groups were as follows: Collembola 379,000; Diplopoda 209,000; Coleoptera 163,000. There were three earthworms Oligochaeta (Terricolae) 23,000 per acre; four Acarina 31,000 per acre; Oligochaeta (Limicolae) 224,000 per acre, and no Chilopoda in the samples.

The number of species of Insects which occurred in the samples was about 30.

The following orders of Insects were represented in the percentages given: Collembola 58.3; Psocoptera 1.2; Thysanoptera 2.4; Hemiptera 1.2; Coleoptera 25.0; Diptera 9.5; Hymenoptera 2.4. The dominant order in number of species present was the Coleoptera with 12 species.

The most abundant species was *Onychiurus armatus* (Tullb.), which made up 42.9 per cent. of the total Insects, the next being *Achorutes purpurescens* Lubb. 7.1 per cent.

The "probable error" of the total number of Insects per acre is  $\pm 36,000$  or  $\pm 5.3$  per cent.

(b) *Ammonium Salts Plot* (No. A 8). Total number of Invertebrates in ten samples 182, or 1,410,000 per acre. Of these 129, or 999,000 per acre, were Insects. In addition a nest of 36 eggs of *Forficula auricularia* was met with, and 27 eggs of *Leptohylemyia coarctata* (209,000 per acre) were also found.

The numbers per acre of the more abundant groups were as follows: Collembola 643,000; Coleoptera 163,000; Acarina 147,000; Diplopoda 31,000; Oligochaeta (Terricolae) 15,000; and Oligochaeta (Limicolae) 341,000. No Chilopoda were found. Of the 19 Acarina found, 17 were hypopi clinging to a single *Forficula auricularia*.

The number of species of Insects which occurred in the samples was about 31.

The following orders of Insects were represented in the percentages given: Collembola 66.4; Orthoptera 2.4; Thysanoptera 2.4; Hemiptera 2.4; Coleoptera 9.6; Diptera 16.0; Hymenoptera 0.8. The dominant order in number of species present was the Coleoptera with 10 species.

The most abundant species was *Onychiurus armatus* (Tullb.), which made up 59.2 per cent. of the total Insects, the next being *Trichocera* larvae 5.6 per cent.

The "probable error" of the total number of Insects per acre is  $\pm 191,000$ , or  $\pm 19.1$  per cent.

(c) *Superphosphate Plot* (No. O 5). Total number of Invertebrates in ten samples 224, or 1,734,000 per acre. Of these 184, or 1,424,000 per acre, were Insects. In addition three eggs of *Leptohylemyia coarctata* (23,000 per acre) were found.

The numbers per acre of the more important groups were as follows: Collembola 1,239,000; Coleoptera 163,000; Diplopoda 31,000; Chilopoda 8000; Acarina 8000; Oligochaeta (Limicolae) 209,000. No Oligochaeta (Terricolae) were found.

The number of species of Insects which occurred in the samples was about 27.



The following orders of Insects were represented in the percentages given: Collembola 87.4; Lepidoptera 0.5; Coleoptera 6.6; Diptera 3.3; Hymenoptera 1.1. The dominant order in number of species present was the Coleoptera with 9 species.

The most abundant species was *Onychiurus armatus* (Tullb.), which made up 77.0 per cent. of the total Insects, the next being *Achorutes purpurescens* Lubb. 4.9 per cent.

The "probable error" of the total number of Insects per acre is  $\pm 285,000$ , or  $\pm 20.0$  per cent.

(d) *Dung Plot* (No. O 1). Total number of Invertebrates in five samples 836, or 12,948,000 per acre. Of these 150, or 2,323,000 per acre, were Insects.

The numbers per acre of the more abundant groups were as follows: Collembola 1,580,000; Coleoptera 418,000; Diptera 294,000; Oligochaeta (Limicolae) 7,155,000; Diplopoda 1,982,000; Oligochaeta (Terricolae) 960,000; Acarina 217,000; Chilopoda 108,000.

The number of species of Insects which occurred in the samples was about 25.

The following orders of Insects were represented in the percentages given: Collembola 68.0; Orthoptera 0.67; Thysanoptera 0.67; Coleoptera 18.0; Diptera 12.0; Hymenoptera 0.67. The dominant order in number of species present was the Collembola with 9 species.

The most abundant species was *Anurida granaria* (Nic.) Tullb., which made up 33.78 per cent. of the total Insects, the next being *Onychiurus fimetarius* (L.) 17.5 per cent.

The "probable error" of the total number of Insects per acre is  $\pm 286,000$ , or  $\pm 12.4$  per cent.

(e) *Dung, Superphosphate and Potash Plot* (No. O 2). Total number of Invertebrates in five samples 610, or 9,448,000 per acre. Of these 143, or 2,215,000 per acre, were Insects.

The numbers per acre of the more abundant groups were as follows: Collembola 1,564,000; Coleoptera 449,000; Diptera 170,000; Oligochaeta (Limicolae) 3,949,000; Diplopoda 2,246,000; Oligochaeta (Terricolae) 558,000; Acarina 170,000; Chilopoda 108,000. Of the 11 Acarina found, three were hypopi clinging to a Diplopod.

The number of species of Insects which occurred in the samples was about 27.

The following orders of Insects were represented in the percentages given: Collembola 70.63; Hemiptera 1.40; Coleoptera 20.28; Diptera 7.69. The dominant order in number of species present was the Collembola with 10 species.

The most abundant species was *Onychiurus fimetarius* (L.), which made up 34.48 per cent. of the total Insects, the next being *Achorutes purpurescens* Lubb. 12.41 per cent., *Anurida granaria* (Nic.) Tullb. 11.03 per cent., and *Atheta* (*Meotica*) *exilis* Er. 10.34 per cent.

The "probable error" of the total number of Insects per acre is  $\pm 303,000$ , or  $\pm 13.5$  per cent.

(f) *Dung and Ammonium Salts Plot* (No. A 1). Total number of Invertebrates in five samples 679, or 10,516,000 per acre. Of these 302, or 4,677,000 per acre, were Insects.

The numbers per acre of the more abundant groups were as follows: Collembola 2,037,000; Diptera 1,533,000; Coleoptera 991,000; Oligochaeta (Limicolae) 4,027,000; Diplopoda 790,000; Oligochaeta (Terricolae) 620,000; Chilopoda 185,000, Acarina 93,000. Of the 99 Diptera found, 91 were larvae of *Trichocera*, which occurred in one sample.

The number of species of Insects which occurred in the samples was about 29.

The following orders of insects were represented in the percentages given: Collembola 45.70; Hemiptera 0.33; Coleoptera 21.19; Diptera 32.78. The dominant order in number of species present was the Collembola with 12 species.

The most abundant species was *Trichocera* sp. (larvae), which made up 30.23 per cent. of the total Insects, the next being *Anurida granaria* (Nic.) Lubb. 18.27 per cent. or, excluding *Trichocera* larvae, 26.19 per cent.

The "probable error" of the total number of Insects per acre is  $\pm 1,180,000$ , or  $\pm 25.0$  per cent.

For the undunged plots taken together the total fauna is 1,451,000 per acre, of which 1,032,000 are Insects. The "probable error" on this total number of Insects is  $\pm 182,000$  or  $\pm 12.5$  per cent.

Taking the dunged plots together the total fauna is 10,971,000 per acre, of which 3,072,000 are insects. The "probable error" on this total number of insects is  $\pm 351,000$  or  $\pm 11.36$  per cent.

## 8. COMPARISON OF THE FAUNA OF THE PLOTS.

In comparing the populations of the different plots, much the most striking difference appears to be between the three not manured with dung, and the three to which dung is applied, irrespective of their treatment as regards artificial manures. The populations per acre of the separate plots and of the undunged and dunged plots considered together, are tabulated in Table I and are also shown diagrammatically in Figs. 1 and 2. The differences in the figures for total Insects are not

Table I.

*Number of individuals in the different orders of  
Insects and other groups in the various plots.*

	Thousands per acre.						Averages	
	Separate plots						Un-	
	O 8	A 8	O 5	O 1	O 2	A 1	dunged	Dunged
Collembola	379	643	1239	1580	1564	2037	754	1727
Orthoptera	—	23	—	15	—	—	8	5
Psocoptera	8	—	—	—	—	—	3	—
Thysanoptera	15	23	8	15	—	—	15	5
Hemiptera	8	23	8	—	31	15	13	15
Lepidoptera	—	—	8	—	—	—	3	—
Coleoptera	163	163	163	418	449	991	163	620
Diptera	62	155	46	279	170	1533	88	661
Hymenoptera	15	8	15	15	—	—	13	5
Total Insects	673	999	1424	2323	2215	4677	1032	3072
Chilopoda	—	—	8	108	108	185	3	134
Diplopoda	209	31	31	1982	2246	790	90	1673
Symphyla	8	—	—	31	31	46	3	36
Araneida	23	8	8	15	31	31	13	26
Acarina	31	147	8	217	170	93	62	160
Oligochaeta	23	15	—	960	558	420	13	712
(Terricolae)								
Oligochaeta	224	341	209	7155	3949	4027	258	5044
(Limicolae)								
Isopoda	15	—	—	—	—	—	5	—
Pulmonata	—	—	—	—	—	31	—	10
Total fauna	1208	1410	1734	12,948	9448	10,516	1451	10,971

Table II.

*Number of species in the different orders in the various plots.*

	Plots						Averages	
	O 8	A 8	O 5	O 1	O 2	A 1	Undunged	Dunged
Collembola	6	7	7	9	10	12	6.6	10.3
Orthoptera	—	2	—	1	—	—	.6	.3
Psocoptera	1	—	—	—	—	—	.3	—
Thysanoptera	1	1	1	1	—	—	1.0	.3
Hemiptera	1	2	1	—	2	1	1.3	1.0
Lepidoptera	—	—	1	—	—	—	.3	—
Coleoptera	10	10	9	7	9	10	9.6	8.6
Diptera	7	7	5	6	6	6	6.3	6.0
Hymenoptera	2	1	2	—	—	—	1.6	—
Chilopoda	—	—	—	1	1	2	—	1.3
Diplopoda	2	2	2	4	5	5	2.0	4.6

significant between the separate undunged and dunged plots, but between these groups of plots taken together the difference is distinctly significant.

This increase in number in the plots receiving dung is noticeable in all groups of Insects and other Invertebrates, as well as in the totals,

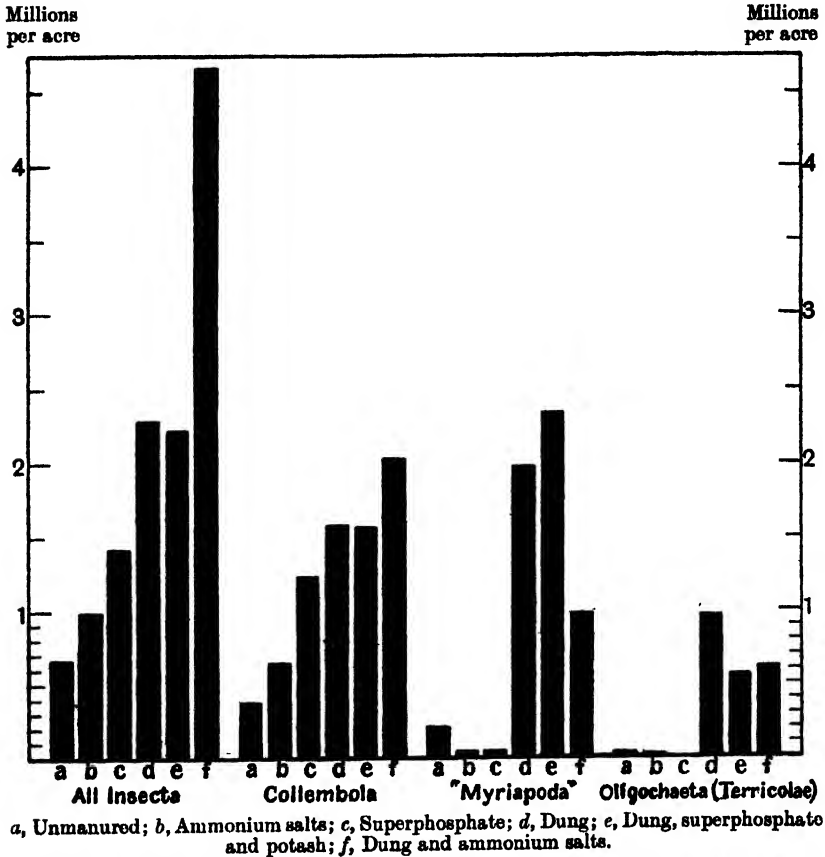


Fig. 1. Number of individuals in the more important groups in the various plots.

and the numbers of species were also increased (Table II). The increase is most marked in the case of the Oligochaeta (Limicolae) which are present in small numbers without dung, but very numerous where dung is applied. Oligochaeta (Terricolae) from being present in very small numbers without dung are numerous with it, Diplopoda are very much increased by dung, and Chilopoda and Elateridae larvae, almost absent

where dung is absent, are present in small numbers where dung is applied.

Of the species of Collembola present *Anurida granaria* and *Onychiurus fimetarius* are much more abundant in the presence of dung, becoming the most abundant species; in the undunged plots *Onychiurus armatus* is much the most abundant species, and is present in considerably greater numbers than it is in the dunged plots. This is the only case observed in which a species was more plentiful in the absence of dung.

100,000  
per acre

100,000  
per acre

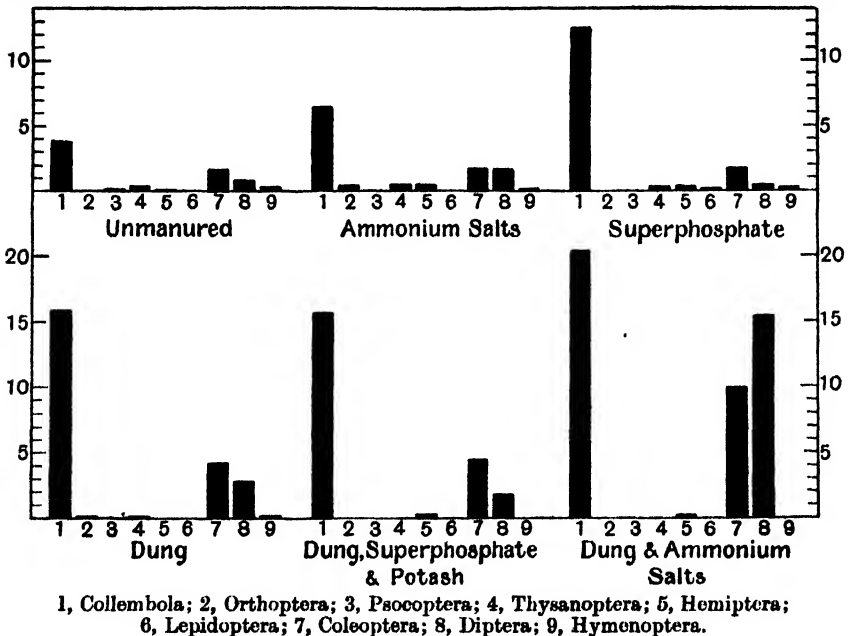


Fig. 2. Number of individuals in the different orders of insects in the various plots.

It is noteworthy also that the injurious Diplopoda were much increased by the application of dung and that Elateridae larvae were also increased.

The occurrence during both autumns over which the investigation extended of eggs of *Leptohylemyia coarctata* is of considerable interest, and has been dealt with more fully elsewhere(7). The larvae of this fly may have been living in the plants of couch grass (*Triticum repens*) which occur in the field, although no such larvae were observed in the few plants of this grass which were examined. Adults of *Atomaria*

*linearis* were the most plentiful Coleopteron and were more numerous in the plots receiving dung, and particularly so in the plot A 1 receiving dung and ammonium salts. This species is known to be a pest of mangolds and in some years has caused considerable damage to the crop in this field. The larva of this species was not recognised, but some unidentified larvae which occurred may have been those of this species.

In connection with the Elateridae larvae it may be mentioned that all the specimens obtained, except one, belonged to the genus *Athous*, while a considerable number obtained from a permanent pasture field adjoining and separated only by a fence, all belonged to the genus *Agriotes*, although there has not apparently been any previous suggestion of either having any particular preference for one type of land rather than another.

#### 9. DISTRIBUTION IN DEPTH.

The samples of soil were taken, as was done previously, in five layers, so that the depth at which the specimens occurred could be recorded. These five layers were, as before: I, surface to one inch below the lowest point of the surface; II, one to three inches; III, three to five inches; IV, five to seven inches; V, seven to nine inches. In taking a sample it was usually quite evident that ploughing had affected the soil to a depth of about five or six inches, a distinct change in the appearance of the soil being noticeable in layer IV, between five and seven inches.

The distribution in depths of the groups—All Insecta, Collembola only, "Myriapoda" and Oligochaeta (Terricolae)—is shown in the diagram (Fig. 3). As was found in the previous investigation, most Insecta and Oligochaeta (Terricolae) were found in the upper five inches of the soil, although the preference for the second level, at a depth of one to three inches, was not so marked, and in several cases most individuals were found in the layer between three to five inches deep.

In some groups, no individuals were found at a greater depth than seven inches, although from the diagram it seems probable that the Insects, Collembola, and "Myriapoda" occur to a greater depth than nine inches, at least in the plots receiving dung.

The percentages of the total Insects at the different depths were:

Unmanured plot:	I 28.0; II 33.6; III 15.7; IV 16.1; V 6.7.
Ammonium salts:	I 19.0; II 45.7; III 21.3; IV 9.3; V 4.7.
Superphosphate:	I 14.1; II 32.9; III 36.0; IV 13.0; V 4.0.
Dung:	I 22.8; II 28.2; III 30.9; IV 9.4; V 8.5.

Dung, superphosphate

and potash: I 17.2; II 41.0; III 22.1; IV 11.0; V 8.2.

Dung and

ammonium salts: I 18.1; II 23.7; III 35.8; IV 14.1; V 8.2.

Owing to the samples from the undunged plots having been taken only during the winter and those from the dunged plots only during the summer, no seasonal variation in distribution is observable, nor is it possible to observe any variation in distribution due to ploughing, as the ploughing was done during the period that samples were being taken from the undunged plots, and the numbers of Insects obtained in each sample from these plots was too small for a useful comparison.

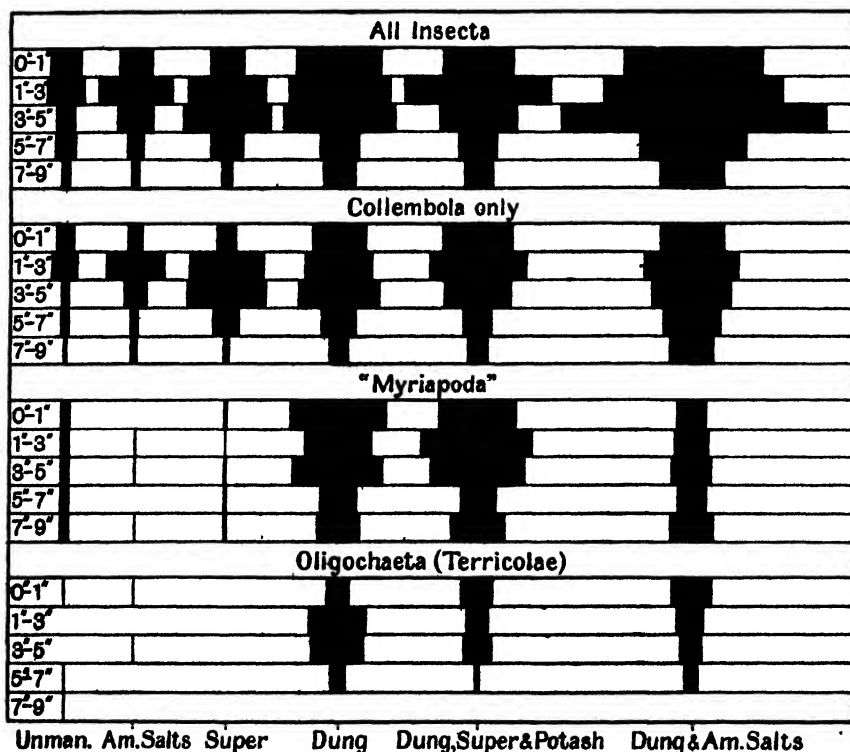


Fig. 3. Distribution in depth of the more important groups in the various plots.

#### 10. COMPARISON WITH OTHER SOIL FAUNA OF ARABLE LAND.

Comparing the insect population of the plots at present under consideration with that of the plots examined previously in Broadbalk field (6), it is at once apparent that the number of Insects present is much

smaller in the present instance. The population of the plots not receiving dung is, in Barn Field, between 500,000 and 1,500,000, as compared with nearly 2,500,000 in the unmanured plot of Broadbalk, the completely unmanured plot of Barn Field containing under 700,000 Insects per acre.

In the case of the dunged plots of Barn Field, the population of two was about 2,250,000 per acre, and of the third 4,750,000, the larger number obtained from the latter plot being due, however, to one unusually rich sample. In Broadbalk the number of insects in the dunged plot was about 7,750,000, so that the numbers of insects in the dunged plots of Barn Field are nearer to that of the unmanured plot of Broadbalk.

The following definitely determined species of Insects were found in Barn Field, which had also been found in Broadbalk:

*Onychiurus fimetarius*, *O. ambulans*, *Isotoma minor*, *Isotomurus palustris*, *Orchesella villosa*, *Heteromurus nitidus*, *Lepidocyrtus cyaneus*, *Forficula auricularia*, *Helophorus nubilus*, *Stenus subaeneus*, *Oxytelus tetracarinatus*, *O. inustus*, *O. nitidulus*, and *Tachyporus hypnorum*.

It is probable that some of the larvae of Coleoptera and Diptera which were not specifically identified were also smaller.

Amongst the "Myriapoda" the following species are common to both fields: *Brachydesmus superus mosellanus*; *Cylindroiulus londinensis* var. *caeruleocinctus*; *Blaniulus guttulatus*; *Archiboreoiulus pallidus*, and *Geophilus longicornis*.

The most interesting new species occurring in Barn Field are *Anurida granaria* and *Onychiurus armatus* which occur in considerable numbers in the dunged and undunged plots respectively. The two species *Atomaria linearis* and *Bourletiella hortensis* are also of interest as they are both pests of mangolds, and the latter caused considerable damage to the young mangold plants during 1926, and, for a short time, was present in very large numbers.

Probably the cause of the differences in soil population between the dunged and undunged plots of Barn Field and those of Broadbalk is the very different amount of cover provided in the two cases by the crop and weeds, and the very different proportions of the year that the fields are bare. In the case of Broadbalk, on which wheat is grown every year, on the dunged plot the crop provides abundant cover through the spring and summer, and in addition to the wheat plants, weeds are present in abundance, and the only cultural operation possible during this time is hand hoeing, which does not cause any very great disturbance of the soil. The only period of the year during which ploughing, etc.,



can be carried out is between the harvest and the autumn drilling for the next year's crop, a period of a month or two. On the unmanured plot of Broadbalk the growth of the crop is not nearly so thick and weeds are less abundant than on the dunged plot, so that not nearly so much cover is provided.

On Barn Field, on which mangolds are grown every year, the crop does not provide more than very scanty cover until well into the summer, and, although weeds are fairly abundant here also, there is nothing approaching the tangle of vegetation which occurs on the dunged plot of Broadbalk. Cultural operations are possible on Barn Field from about November until the next year's crop is sown, about the end of April, and, during most of the summer, hoeing—both by horse and by hand—is carried out at intervals.

From the foregoing comparisons it appears that the soil fauna is influenced largely by the use of dung as manure, and by the amount of vegetation on the ground, and to a very small extent, if at all, by the use of artificial manures.

Since the previous paper by the present writer was published in 1922, an important paper on the soil fauna near Aberystwyth by Miss M. Thompson<sup>(9)</sup> has been published. In this paper the author gives an account of an investigation of the soil fauna of pasture land, ungrazed grassland, land recently ploughed, and of cultivated land, of which the latter is most nearly comparable with the area discussed in the present paper, although the manuring and cropping have not been the same from year to year, the field thus being more typical of normal arable land than Barn Field. This irregularity of treatment has probably prevented the manuring from having any marked effect.

The numbers per acre calculated from Miss Thompson's data give about 4,500,000 Insects per acre, of which over 94 per cent. were Collembola. This total is greater than that in two of the three dunged plots examined in Barn Field but the number of Insects other than Collembola is not so great at Aberystwyth as in the dunged plots of Barn Field, probably owing to the heavy and regular dressings of dung on the Barn Field plots: the number of Insects other than Collembola at Aberystwyth is not greatly in excess of the numbers in the undunged plots of Barn Field. Possibly the much greater numbers of Collembola at Aberystwyth may be due to the open and porous nature of the soil there, compared with the high percentage of clay in the Barn Field.

The number of *Oligochaeta* which would come under the grouping "*Oligochaeta* (*Terricolae*)" used in the present paper, is rather less at

Aberystwyth than in the dunged plots of Barn Field, and of the group "*Oligochaeta* (*Limicolae*)" is very considerably less. This again is directly attributable to the much smaller amount of dung the ground there had received.

The *Diplopoda*, while more numerous in the Aberystwyth area than in the Barn Field undunged plots, are considerably less numerous than in the dunged plots; even on an occasion when they were causing appreciable damage at Aberystwyth their number was less than their average number on two of the dunged plots.

#### SUMMARY.

Samples of soil were taken from six of the plots of Barn Field on the farm of the Rothamsted Experimental Station, and the Insects and other Invertebrates found therein are recorded together with the approximate depth at which they occurred.

On these plots one receives no manure, one superphosphate only and one ammonium salts only, while of the other three, all of which receive dung, one receives superphosphate and potash, and another ammonium salts in addition.

The total number of Insects and other Invertebrates per acre in the undunged plots were 1,208,000, 1,410,000 and 1,734,000 respectively. Of these 673,000, 999,000 and 1,424,000 respectively were Insects. Similarly in the dunged plots the total numbers of Insects and other Invertebrates per acre were 12,948,000, 9,448,000 and 10,516,000 respectively, and of these 2,323,000, 2,215,000 and 4,677,000 respectively were Insects.

Each sample was taken in five layers so that it was possible to record the approximate depth at which each individual occurred. The greatest number, both of Insects and of other Invertebrates, occurred in the upper five inches of the soil.

From the figures obtained in this investigation it appears that artificial manures have little or no effect on the soil fauna, while the effect of dung in increasing the numbers and the number of species of the fauna is very considerable.

## LITERATURE.

(Additional to that mentioned in (6) below.)

- (1) BUCKLE, P. (1921). Preliminary survey of the soil fauna of Agricultural land. *Ann. App. Biol.* viii, Nos. 3 and 4, 135-145.
- (2) — (1923). Ecology of soil insects on agricultural land. *Journ. Ecol.* xi, No. 1, 93-102.
- (3) CAMERON, A. E. (1925). Soil Insects. *Science Progress*, xx, No. 77, 92-108.
- (4) MCCOLLOCK, J. W. and HAYES, W. P. (1922). The reciprocal relation of soil and insects. *Ecol.* iii, No. 4, 288-301.
- (5) — (1923). Soil temperature and its influence on white grub activities. *Ecol.* iv, No. 1, 29-36.
- (6) MORRIS, H. M. (1922). Insect and other invertebrate fauna of arable land at Rothamsted. *Ann. App. Biol.* ix, Nos. 3 and 4, 282-305.
- (7) — (1925). Note on the Wheat Bulb Fly (*Leptohylemyia coarctata* Fall.). *Bull. Ent. Res.* xv, No. 4, 359-360.
- (8) PILLAI, S. K. (1921). Beiträge zur Kenntnis der Fauna der Waldstreu. Kiefern-streu-Untersuchungen. *Zeits. angew. Ent.* viii, No. 1, 1-30.
- (9) THOMPSON, M. (1924). The soil population. An investigation of the biology of the soil in certain districts of Aberystwyth. *Ann. App. Biol.* xi, Nos. 3 and 4, 349-394.
- (10) VON PROTTE, J. F. (1925). Beiträge zur Kenntnis der Fauna der Waldstreu. Fichtenstreu-Untersuchungen. *Zeits. angew. Ent.* xi, No. 1, 35-54.

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## NOTE ON THE ACTIVITIES OF HUMBLE BEES (*BOMBUS*) IN NORTH WALES

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IN August 1923 the writer published a short article in the *Entomologists' Monthly Magazine* giving a brief account of the activities of various species of *Bombi* noted during the period 1913-21, chiefly from records gathered in North Wales in 1919-21, together with a few from Mid-Wales, and other parts of the west of England. These notes have since been extended (1923-26) by a further mass of records from North Wales (and a few from West Herefordshire and Montgomeryshire).

As a result it is believed that a fairly complete idea has been gained of the work of the more abundant species, throughout their active periods, at any rate for North Wales, and adjacent regions. The less abundant mountain species have received less attention than the more abundant lowland forms. To tabulate the full activities of each species would require more space than seems commensurate with the subject and an attempt is made to combine the chief information as far as possible in one table. The data presented was gathered, for the most part during field work in ordinary agricultural land, gardens, etc., and no type of cultivation was given predominance.

All records relate to actual flower visits, including the dates given for "First noted" and "Last noted," which do not refer to nest-hunting "queens," etc.

The following species have been noted during the work, and are given below together with some general remarks, and a list of the flowers visited (other than those of chief economic value, which will be found in the table appended). The records are not given in botanical order but in sequence of seasonal observation.

(1) *BOMBUS LAPIDARIUS*. Common everywhere. Recorded from a total of 27 flower species (economic and non-economic). Gorse, Rosemary, Rhododendron, Garden Anemone, Dog Violet, Apple, Daisy, Bluebell, Buttercup, Laburnum, Escallonia, *Vicia sepium*, Self Heal, Yellow Rattle, *Centaurea nigra*, Hieracium, Wild Thyme, *Leontodon autumnale*, Rest-harrow, Red Bartsia, various meadow Compositae, Brassicas.

(2) *B. TERRESTRIS* and (3) *B. LUCORUM*. These two species are both very abundant, and in their times of appearance and activities show great similarity. *B. terrestris* has been recorded from 45 flower species, and *B. lucorum* from 54.

The flowers visited by the latter include Rosemary, Heaths (Garden), *Berberis* spp., Mahonia, Gorse, Dandelion, Red Ribes, Sallows, Bluebell, Sycamore, Ground Ivy, Iris, Escallonia, Charlock, Lousewort, Ox-eye Daisy, Sage (Garden), Caper Spurge, Sheep's Scabious, Fuchsia, *Hieracium* spp., *Helianthemum* sp., *Campanula* spp., Burdock, Figwort, Thistles, Bartsia, Snowberry, Scabious, Privet, Wood Sage, *Solanum jasminoides*.

(4) *B. SORRÖENSIS*. Recorded in small numbers, July to September, on Dandelions, Blackberry, and *Centaurea scabiosa*.

(5) *B. PRATORUM*. Common in North Wales, and recorded as visiting 36 species of flowers, the non-economic species being *Berberis* spp., Wood Anemone, False Strawberry, Heaths, Dandelion, Bluebell, Laburnum, Columbine, Marigold, Comfrey, Yellow-Rattle, Escallonia, Snowberry, Thistles, *Hypericum* sp., Sheep's Scabious, Veronica (cultivated), Rest-harrow, Antirrhinum, *Cotyledon umbilicus*, Charlock, Sea Holly, *Centaurea nigra*.

(6) *B. JONELLUS*. A rare mountain species in North Wales, a few having been obtained from June to August on *Jasione montana*, *Erica cinerea*, and *E. vagans*.

(7) *B. LAPPONICUS*. Not a common species. Recorded from May 27th to end of August, visiting *Cardamine pratensis*, White clover, *Pedicularis sylvatica* (once each) and fairly common on Blackberry and *E. vagans*.

(8) *B. RUDERATUS*. The only specimens referable to this species are several melanic ♂♂ collected about Dyserth and Rhyl, August 10th, 1923. These were entirely black with the exception that "the beards of the mandibles are rust-coloured" as stated by Sladen (2), p. 175, also a few pale hairs and a tendency to greyish-brown on the tail, more especially beneath. No females or workers were obtained although sought for.

(9) *B. HORTORUM*. Common, and reported from 23 flower species. Primrose, Pulmonaria, Bluebell, Columbine, Hawthorn, Escallonia, Red Campion, Horse-Chestnut, Sage (Garden), *Convolvulus major*, Foxglove, *Centaurea nigra*, Self-Heal, *Stachys germanica*, *S. sylvatica*, Wood Sage, Antirrhinum (Garden), Burdock, Fuchsia, Honeysuckle, Bell-Heather, Herb-Robert Thistles, *Tropaeolum indicum*, Common Hemp Nettle.

(10) *B. DISTINGUENDUS*. A fine "queen" was taken near Bangor, June 22nd, 1924, crawling on a road, during cold, windy weather.

(11) *B. LATREILLELLUS*. Only one specimen was taken, July 11th, 1920, on white Clover near Bethesda.

(12) *B. DERHAMELLUS*. Local, recorded April-July on Dog Violets, Yellow Rattle, and *Lathyrus pratense*.

(13) *B. AGROBUM*. The most abundant species in North Wales, found throughout the season, and later than any other species, being noted as visiting *Galeopsis* near Bethesda at 700 feet on November 18th, 1926. Collected from 70 flower species, including Herb Robert, Wallflower, Dog Violets, Dandelions, Ground Ivy, Lungwort, Bilberry, Garden Brassicas, Lady's Smock, *Lathyrus* sp., *Valeriana pyrenaica* *Centaurea* sp., Comfrey, *Ajuga reptans*, Purple Dead-nettle, Blue-bell, Lesser Spearwort, Dog Rose, Garden Roses, (White) Rhododendrons, Escallonia, Horse-Chestnut,

*Mimulus* sp., Sage (Garden), Foxglove, Red Campion, *Vicia cracca*, *Lotus major*, Shirley Poppy, Sheep's Scabious, Burdock, Spear Thistle, *Calamintha*, Hemp Nettle, Wood Sage, Self Heal, Wood Betony, Antirrhinum (Garden), Purple Loose-strife, Fuchsia, *Vicia orobus*, Gorse, Field Scabious, *Stachys sylvatica*, Red Bartsia, White Dead-nettle, Hollyhock.

(14) *B. HELFERANUS*. Local, and appears late, not being recorded prior to July 16th. In addition to a few economic records (see Table) it has occurred on Hollyhock and *Centaurea nigra*.

(15) *B. MUSCORUM*. Only one definite record, a queen taken by Mr W. Maldwyn Davies, B.Sc. This specimen was pollen-collecting on Lilies, Bangor, June 18th, 1924.

In the table the following abbreviations are used:

c. = common; v.c. = very common; f.c. = fairly common; s. = several records in different years.

(1), (2), etc., indicate only that number of records.

The other figures denote months.

#### GENERAL NOTES.

In 1923 the spring weather was cold, with practically no early bursts of heat and very few ♀♀ *Bombi* were noted until the warm days of May 7th–10th, when great numbers were seen. In marked contrast was April 9th, 1926, when "queens" of six species were abundant and active.

A ♂ and ♀ of *B. hortorum* were collected in copula on herbage on the headland of a field of mangolds, etc., at 2 p.m. on Sept. 10th, 1926, in bright hot weather, in Carnarvon.

As regards "robbing" the only two instances noted both relate to *B. pratorum*, the first of workers nectar sucking from numerous perforated flowers of Comfrey, at Aber, May 11th, 1926, and the second at Bangor, June 13th, 1926, when the flowers of Broad Beans were being visited at 9.30 p.m. (summer time). In this case the bases of all the corollas were badly damaged by perforations made on their upper surface. In neither instance was *pratorum* observed actually doing the damage.

*B. pratorum* was likewise observed nectar sucking from the extra floral nectaries of Broad Beans, in a Bangor garden, June, 1926. Williams (3) dealing with red clover at Aberystwyth concluded that not more than 4–5 per cent. of the total bees present were "robbers."

*B. hortorum* was observed visiting and nectar sucking from "thrum" eyed flowers of Primrose in April, 15 being visited in rapid succession on two contiguous roots growing on a rockery amongst many other plants.

Further instances noted of bees passing from one flower species to another are as follows: (1) *B. lapidarius* ♀, *Bellis perennis* to *Viola* sp. (dark blue). (2) *B. hortorum* (at 1000 ft. in Montgomeryshire), Foxglove

*Table showing work of Bombi.*

Fruit, Crops and Vegetables

Species of Bee	Apple	Pear	Plum	Cherry	Rasp- berry	Black- berry	Goose- berry	White Clover	Red Clover	Alsike	Lotus	Broad Bean	Vetch	Runner Bean	Marrow	Remarks
<i>B. lapidarius</i>	4 s.	—	—	—	—	7-8 f.c.	—	6-7 f.c.	5 s.	—	6-8 s.	—	6	—	—	Common everywhere. First noted Apr. 6th. Last noted Sept. 2nd. Most abundant June-Aug.
<i>B. terrestris</i>	4-5 f.c.	5	4 c.	4 c.	6 c.	7 c.	4 f.c.	6	6-7 (1)	7	—	6-7	8 v.c.	7-8 c.	9 s.	Common everywhere. First noted Apr. 1st. Last noted Sept. 10th. Most abundant Apr.-Aug.
<i>B. lucorum</i>	5-6 f.c.	—	4 f.c.	4 c.	5-6 c.	7 c.	4 s.	6-7-8 f.c.-s.	6	6-7 c.	6	6-7 v.c.-s.	8 v.c.	7-8	9 s.	Common everywhere. First noted Apr. 1st. Last noted Sept. 25th. Most abundant Apr.-Aug.
<i>B. pratorum</i>	5 s.	—	4 s.	4 (1)	5-6 c.	6-7 c.	3 (1)	—	—	—	7	—	—	6	—	First noted Apr. 1st. Last noted Aug. 17th. Most abundant Apr.-July.
<i>B. hortorum</i>	—	—	—	—	7 c.	7 c.	—	7 s.	7 s.	—	—	6 f.c.	—	—	9	First noted Apr. 25th. Last noted Oct. 14th. Most abundant June-July.
<i>B. agrorum</i>	4-5	—	4 (1)	—	5 f.c.	6-7-8 c.	—	6-7 c.	6-9 c.	6-7 v.c.	6-7 v.c.-(1)	6 f.c.	7	7 c.	9	Very abundant. First noted Apr. 6th. Last noted Nov. 10th. Most abundant May-Sept.
<i>B. cheramelus</i>	—	—	—	—	5	—	—	7	7	—	—	—	—	—	—	Local. Apr.-Aug.
<i>B. helicanus</i>	—	—	—	—	—	8 s.	—	7	9 (1)	—	—	—	—	—	—	Local. July 16th-Sept. 24th.

to *Stachys sylvatica* (several) and on to *Erica cinerea*. (3) *B. agrorum*, worker, *C. nigra* to *Hierachium* sp. This species was also observed to visit colonies of *Psylla crataegi* on Hawthorn, near Bangor, June, 1926, in order to lap up "honey dew."

#### SUMMARY.

1. A short account is given of the species of Humble Bees (*Bombi*) observed by the writer in North Wales and adjacent districts during the period 1919-27, with notes on their relative abundance and seasonal activities.

2. The chief economic plants visited by each species are listed, together with brief mention of those cultivated and wild flowers most favoured.

#### LITERATURE.

- (1) WALTON, C. L. (1923). Observations on the Genus *Bombus*, etc., in North Wales. *Ent. Monthly Mag.* 2nd Ser. VIII, 271-275.
- (2) SLADEN, F. W. L. (1912). *The Humble Bee*.
- (3) WILLIAMS, R. D. (1925). Studies concerning the Pollination, Fertilisation and Breeding of Red Clover. *Welsh Plant Breeding Station*, Ser. H. No. 4.

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# ON THE PAIR OF SO-CALLED SENSORY PITS OF THE NINTH ABDOMINAL SEGMENT OF THE WIREWORM (*AGRIOTES OBSCURUS* LIN.) WITH ADDITIONAL NOTES ON THE INTERNAL ANATOMY

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(With 9 Text-figures.)

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## 1. INTRODUCTION.

THIS paper represents an endeavour to ascertain the structure and the function of the curious pair of stigma-like depressions or pits (Fig. 1, *PT*) which are to be found on the ninth abdominal segment of the wireworm (*Agriotes obscurus* Lin.). Earlier writers erroneously referred to them as spiracles but later authors have described them either as muscular impressions or sensory pits.

Horst(6), 1922, suggests that they may represent a modified ninth pair of stigmata and points out that nowhere in the literature is he able to obtain any guidance concerning the significance and embryological development of these structures.

Whilst examining the voluminous literature which has accumulated concerning such an important economic species as the wireworm the writer was rather surprised to note that practically nothing has been recorded concerning the internal anatomy of the wireworm nor indeed of Elaterid larvae generally.

For instance, Horst (6) in his fairly elaborate paper on the morphology and biology of the Elateridae gives no account of the internal anatomy of the larvae, although the external appearance is extensively treated. Consequently some additional information has been included in this paper on the internal anatomy of *Agriotes obscurus* (larva).

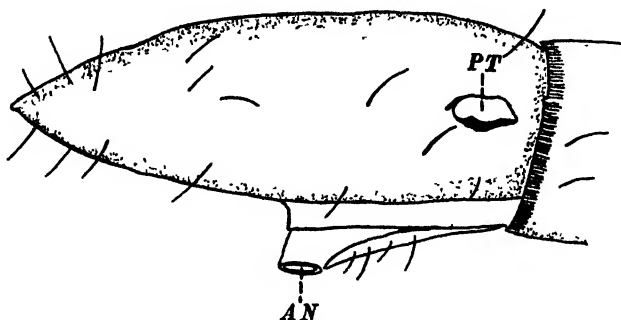


Fig. 1. The lateral view of the last or ninth abdominal segment of the larva of *Agriotes obscurus*.  $\times 50$ . AN, anus; PT, pit.

I desire to thank Prof. J. S. Gardiner, F.R.S., for the facilities he has afforded me to carry out this work in his laboratory.

Acknowledgements are due to Mr A. Rymer Roberts, M.A., who determined the instars of some of the wireworms worked on; to Dr Keilin, who examined my sections of the malpighian tubes; and also to Mr F. R. Petherbridge, of the School of Agriculture, Cambridge, who provided me with some material.

## 2. TECHNIQUE.

The chief technical difficulty encountered in attempting to obtain sections through the region of the pits or indeed through any part of a wireworm is the well-known toughness and thickness of the cuticle.

The following method was devised to obtain sections through the pits.

The larvae were fixed in either Carnoy's (No. 2 formula) or Bouin's fluid and the ninth abdominal segment then cut away and embedded in paraffin wax using ordinary methods. The embedded segment was then cut out of the wax and the two parts of the segment containing pits cut away, care being taken to chip away as much of the integument as possible without injuring the pits and the soft structures associated with them. The chitinous wall of the pits is much thinner than that of the cuticle of the general body surface and after being re-embedded good

sections were obtained. Whole mounts were also made of the pits and nitric acid proved a useful and rapid decoloriser of the chitinous parts.

For the examination of the internal anatomy of the larva dissections were made and the parts studied were both mounted whole and sectioned. The alimentary canal and associated structures were best cut at  $6\mu$  thick but the best sections of the chitinous parts were obtained from

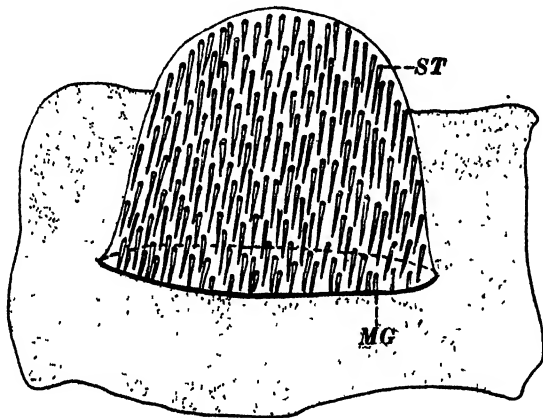


Fig. 2. One of the pits on the last abdominal segment of *A. obscurus* (larva) viewed from the inner aspect and rendered partly transparent with nitric acid.  $\times 630$  diams. MG, thickened margin of pit; ST, setae lining the outer surface of the pit wall.

sections  $10\mu$  thick. Delafield's iron haematoxylin proved sufficient for nearly all staining purposes and safranin was occasionally used as a counter stain.

All sections and dissections were made from larvae of the last instar. I am indebted to the late Dr C. L. Withycombe for securing for me a supply of reliable indicators which were used to test the reaction of the various parts of the alimentary canal and its contents.

### 3. THE "SENSORY" PITS.

The external appearance of these two depressions or pits which are to be found on the last abdominal segment of the larva of *Agriotes obscurus* has been adequately described by Roberts (8).

This worker in describing the ninth abdominal segment of the wireworm (*A. obscurus*) writes: "The ninth abdominal segment is considerably longer than the preceding one, conically paraboloid (Beling(1)) with a pair of large open pits margined with brown situated near the anterior margin of the tergite. In life they are nearly round but contract at the

sides to an elongated oval shape when preserved in spirit. Their margin of stout brown chitin is somewhat raised above the general area of the tergite on either side. Within the pits are lined with a pale membrane which bears numberless minute dark hairs arranged over the entire surface. Similar fine hairs are also found on the inner surface of the chitinous rim just mentioned. In consequence of the presence of these hairs situated in pits the latter are presumed to have a sensory function though what it may be has not been ascertained. Formerly they were supposed to be spiracles but more recently they have been referred to as muscular impressions by Henriksen(5) and Schiodte(9).

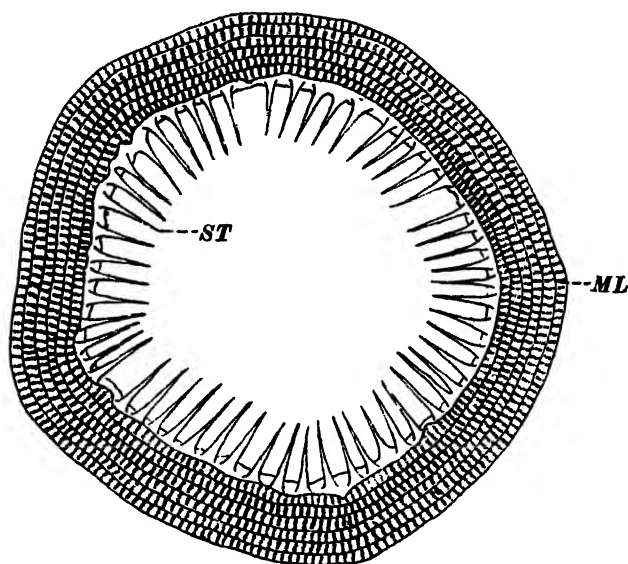


Fig. 3. A transverse section of a pit.  $\times 780$  diams. *ML*, muscles which completely surround the inner surface of the pit wall; *ST*, setae lining the outer surface of the pit wall.

An examination of sections of the pit wall show that the hairs (Figs. 2 and 3, *ST*) lining its outer surface are setiform in shape and arise from the general surface of the cuticle. Moreover, they do not possess a nervous supply at their bases and consequently cannot be differentiated from ordinary body hairs nor grouped with any known type of sensillae.

No other type of structure which is associated with sense reception in insects was found in the region of the pits and neither by dissection nor by examination of sections was any direct nerve supply traced to the pits. Lining the inner surface of the pit wall and closely appressed to it is a thick layer of muscular tissue (Fig. 3, *ML*). From the foregoing

it appears that if these pits and their setae possess any function of a sensory nature it must be of a very diffuse and unspecialised type. It is possible, keeping in view the fact that the cuticle of the pit wall is thinner than that of the general body integument, that the animal may be sensitive to differences of pressure in the region of the pit of which the greater resisting power of the rest of the cuticle would otherwise render it oblivious.

From this study of their structure, however, the term sensory pits does not seem to be justified and the evidence seems to point to the fact that they are connected in some way with the functions of the muscles and are very probably rather large muscular impressions.

In reference to the opinion of Horst<sup>(6)</sup> that the pits may represent a modified ninth pair of spiracles it may be stated that no structures were found which would tend to support such a view, but as my sections were made entirely from larvae in the last instar a study of earlier stages might prove useful.

#### 4. THE ALIMENTARY CANAL.

The alimentary canal is a practically straight tube which is remarkably free from the appendicular glands often associated with the gut of both larval and adult insects.

A very careful search enables the writer to state definitely that those structures usually referred to as salivary glands in insects are absent. This state of affairs is not an unusual occurrence in both larval and imaginal Coleoptera. Gastric coeca also are not found and histological evidence suggests (Fig. 6) that the digestive fluids are secreted entirely by the epithelial lining of the mesenteron.

Immediately behind the oral opening the gut swells slightly to form a pharynx (Fig. 4, *PH*) and thereafter narrows and is continued as the oesophagus (Fig. 4, *OE*) to about the posterior region of the second thoracic segment where the calibre of the tube again increases sharply to form the mesenteron (Fig. 4, *MS*). The inner surface of the wall of the oesophagus projects into the lumen to form four longitudinal ridges extending the whole of its length (Fig. 5, *LR*). They are supplied with muscular elements from the circular layer (Fig. 5, *CM*) and by their movements assist the passage of food along the gut.

The longitudinally arranged muscle bands are very powerful, especially in the region of the pharynx.

The oesophagus at its junction with the mesenteron projects somewhat into the lumen of the latter (Fig. 4, *EV*), forming a kind of valve.

The mesenteron is a tube of fairly uniform calibre extending from the hinder part of the second thoracic segment to the region of the seventh or eighth abdominal segment and varies from eight to ten millimetres in length. Stained surface preparations of the mesenteron show externally a series of longitudinal muscle bands beneath which may be seen a layer of circular muscle fibres. Fig. 6 represents a transverse section of the mesenteron in its posterior region. Internally the epithelial cells are seen in both the secretory phase (*AE*) and the quiescent phase (*IE*). When actively secreting the cells elongate in a radial direction and vesicles containing fluid are "nipped off" and mingle with the alimentary matter lying on the lumen of the gut. The presumptive evidence is very strong that those vesicles contain the digestive ferments. Numbers of these vesicles could be observed lying in the lumen of the mesenteron (Fig. 6, *VS*) and the digestive ferments probably pass out by a process of dialysis and mix with the food material. There is no peritrophic membrane of any type in the gut of the wireworm (*Agriotes obscurus*).

The epithelial lining is thicker and usually more active in the middle region of the mesenteron and there is a greater tendency for it to be thrown into folds. There does not appear to be any differentiation of the epithelium into absorbent and secretory cells although the less active epithelial cells near the posterior end of the mesenteron may have a specialised absorbing function. The outer ends of the epithelial cells rest on a basement membrane (Fig. 6, *BM*). Groups of small cells with darkly staining nuclei (Fig. 6, *CC*) are to be seen at intervals beneath the epithelium.

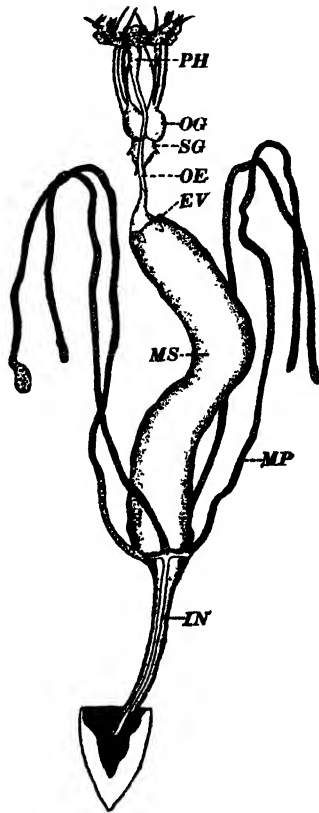


Fig. 4. The alimentary canal of *Agriotes obscurus* (larva) with part of the central nervous system.  $\times 50$ . *EV*, oesophageal valve; *IN*, small intestine; *MP*, malpighian tube; *MS*, mesenteron; *OE*, oesophagus; *OG*, supra-oesophageal ganglion; *PH*, pharynx; *SG*, sub-oesophageal ganglion.

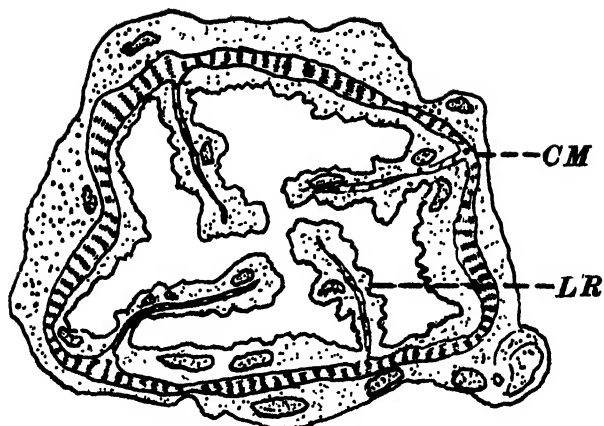


Fig. 5. A transverse section of the oesophagus showing the four longitudinal ridges which project into the lumen of the tube throughout its length.  $\times 600$  diams. *CM*, circular muscular layer; *LR*, longitudinal ridge.

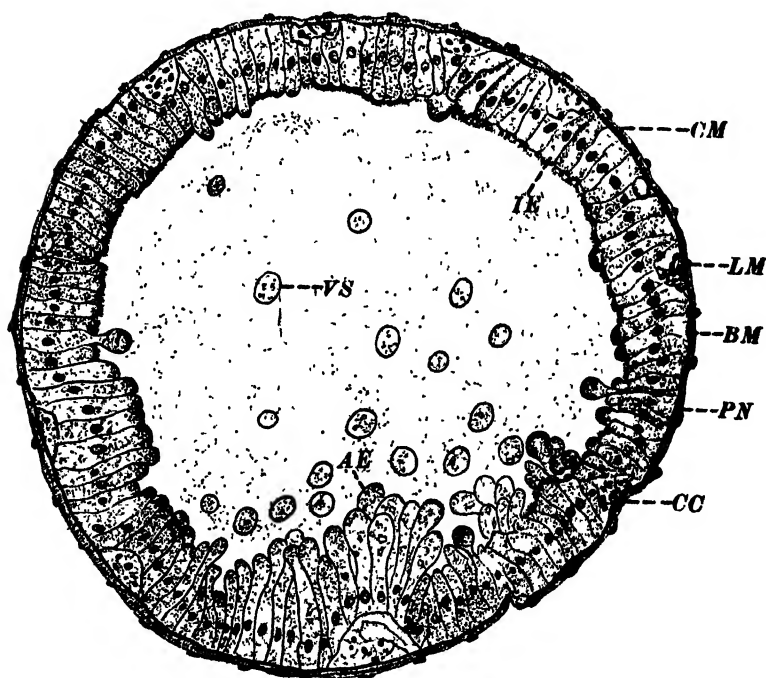


Fig. 6. A transverse section of the mesenteron near its posterior extremity showing the epithelial cells in both secretory and resting phases.  $\times 300$  diams. Drawn from sections  $6\mu$  in thickness. *AE*, epithelial cells in active secretory phase; *BM*, basement membrane; *CC*, crypt cells; *CM*, Circular muscle layer; *IE*, epithelial cells in the resting phase; *LM*, longitudinal muscle layer; *PN*, peritoneal layer; *VS*, a vesicle from a secretory epithelial cell.

Similar groups of cells were described by Gehuchten (4) in *Ptychoptera contaminata* and this writer named them crypt cells because they occurred in the hollows beneath the folds of epithelium. Gehuchten regarded them as centres of regeneration for epithelial cells.

Covering the outer surface of the mesenteron is a thin sheet of connective tissue which corresponds to the peritoneal layer of some other insects (Fig. 6, *PN*). The contents of the mesenteron when dissected out in double distilled water immediately after killing gave an alkaline reaction to Bromothymol blue and the pH lay between seven and eight.

The walls of the mesenteron gave a similar reaction. The hindgut

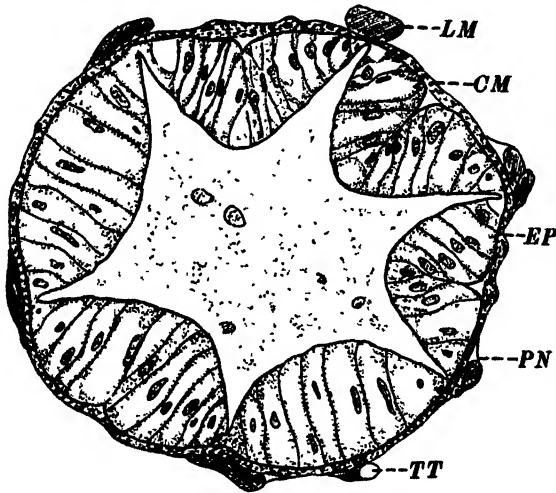


Fig. 7. A transverse section of the hindgut in the region of its middle third.  $\times 500$  diams. *CM*, circular muscle layer; *EP*, epithelium; *LM*, longitudinal muscle band; *PN*, peritoneal layer; *TT*, tracheal tube.

measures from 3 to 5 mm. in length and there is no differentiation into intestinal and rectal regions (Fig. 4, *IN*). The walls are composed internally of a layer of large epithelial cells which are grouped into six ridges for the greater part of its length (Fig. 7, *EP*). There is, however, a region midway along the hindgut where two pairs of ridges gradually run together, thus reducing the number to four, two large ridges alternating with two small ones. Posteriorly again the two large ridges split to re-form the original six ridges but these become very irregular in shape and size near the anal region.

The nuclei of the epithelial cells vary in shape but are usually somewhat elongated with the long axis radially directed.



The epithelium rests on a basement membrane and external to the latter is a layer of striated circular muscle fibres. Six comparatively large muscle bands lie outside the circular muscles and each of these bands alternates with an internal ridge of epithelium. Enveloping all these structures is a thin lining of connective tissue (Fig. 7, *PN*). The chitinous intima which lines this region of the gut is thicker in the anal region. Anal and rectal glands are absent. The alimentary canal opens to the exterior through a longitudinal slit on an anal papilla (Fig. 1, *AN*) situated on the anterior ventral surface of the last abdominal segment.

##### 5. THE MALPIGHIAN TUBES.

The malpighian tubes in both *Agriotes obscurus* and *Athous haemorrhoidalis* consist of four rather long yellow tubes whose distal extremities terminate freely (Fig. 4, *MP*), there being no secondary attachment to the gut or to each other such as occurs in the larvae of certain other Coleoptera. Occasionally the tubes are coloured very dark brown to black in irregular patches which vary in length from 1 to 3 mm.

An examination of sections of the darkened parts of the tube revealed that their lumina were completely occluded with a mass of non-crystalline excretory matter which gave the characteristic colour to that part of the tube (Fig. 8).

The malpighian tubes when dissected out in double distilled water and immediately transferred to indicators gave a decided acid reaction in bromothymol blue but was alkaline in bromophenol blue. The latter indicator has a range of pH 2.8-4.6. The hindgut reacted in a similar manner to these indicators. The cells lining the lumen of the malpighian tubes near their proximal ends are smaller and more regular in shape than those nearer the distal extremities and a considerably greater number of the former are required to line the periphery of the tube. The muscular layer of the hindgut sends some of its fibres along the malpighian tubes and the latter may be capable of some independent motion, but this was not observed.

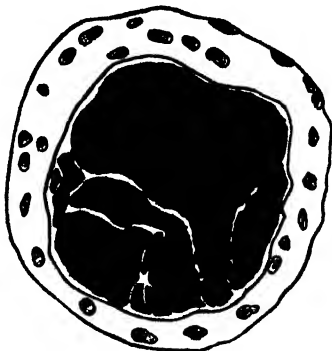


Fig. 8. A transverse section of a malpighian tube showing the lumen occluded with black excretory matter. Drawn from sections 15  $\mu$  thick.  $\times 800$  diams.

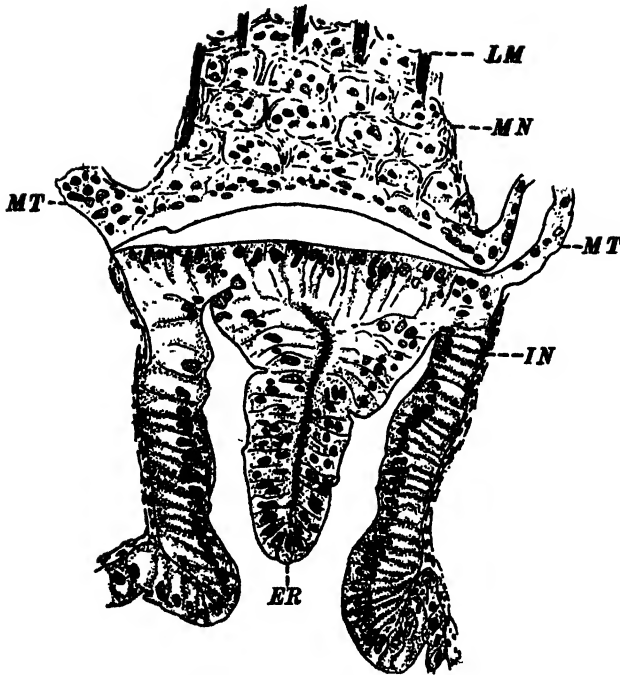


Fig. 9. An oblique section through the alimentary canal at the junction of the mesenteron and the hindgut showing the opening of one of the malpighian tubes into the gut. Drawn from sections  $6\mu$  thick.  $\times 300$  diams. *ER*, an epithelial ridge of the small intestine; *MN*, mesenteron; *MT*, malpighian tube; *LM*, longitudinal muscle of mesenteron; *IN*, small intestine.

#### 6. THE CENTRAL NERVOUS SYSTEM, DORSAL VESSEL AND FAT BODY.

The central nervous system consists of the usual pair of supra-oesophageal ganglia (Fig. 4, *OG*) which lie above the oesophagus in the prothorax and send forward numerous nerves into the cephalic region.

The sub-oesophageal ganglia (Fig. 4, *SG*) whose double natures are not obvious lie close up to the supra-oesophageal ganglia.

The connectives which join the three thoracic ganglion masses together are double but those connecting the eight abdominal masses appear as single cords. The connective joining the last two abdominal nerve centres is very short and there appears to be a tendency towards coalescence. The dorsal vessel was also observed and consists of a non-septate tube extending nearly the whole length of the animal.

The fat body consists of narrow leaf-like folds sometimes of extraordinary length which may entirely disappear in larvae which have been starved for some considerable time.

## SUMMARY.

The structural evidence obtained from the study of the pits on the ninth abdominal segment of *Agriotes obscurus* favours the view that they are a pair of muscular impressions. The hairs lining the pit walls do not possess a nervous supply nor was any special nervous supply traceable to the pits as a whole. No structures which have been associated with sense reception in insects were found in the neighbourhood of the pits. There was no evidence that the pits are a modified pair of spiracles as was suggested by one writer (6).

With regard to the alimentary canal there are no salivary glands. The oesophagus possesses four ridges which project into its lumen and their muscular nature suggests that they are capable of independent movement.

The mesenteron is devoid of gastric coeca and does not contain any peritrophic membrane of any type.

The digestive fluid is secreted in vesicles which are budded off from the epithelial cells lining the midgut. The contents of the mesenteron has a pH of between seven and eight which is similar to the reaction of its cell walls. The hindgut is a straight tube without clearly marked intestinal and rectal regions and there are no glands of any kind in this part of the gut.

The malpighian tubes are four in number and are generally of a yellow colour, but it is not unusual to find blackened patches in the tubes which are due to dark masses of excretory matter blocking the tube.

The dorsal vessel is non-septate.

Besides the supra- and sub-oesophageal ganglia there are 11 nerve masses in the central nerve chain—three thoracic and eight abdominal.

The masses of fat body are characterised by their leaf-like shape and great length.

## BIBLIOGRAPHY.

- (1) BELING, TH. (1883, 1884). Beitrag zur Metamorphose der Käferfamilie der Elateriden. *Deutsche Entomologische Zeitschrift*, xxvii, 129-144, 259-304, und xxviii, 177-216.
- (2) BERLESE, A. (1909). *Gli insetti*, pp. 559-714. Milan.
- (3) FORD, G. H. (1917). Observations on the larval and pupal stages of *Agriotes obscurus* Linn. *Ann. App. Biol.* iii, 97-114 (2 pls. and 1 text-fig.).
- (4) GEHUCHTEN, VAN. (1890). Recherches histologiques sur l'appareil digestif de la *Psychoptera contaminata*. *La Cellule*, No. 6.
- (5) HENRIKSEN. (1912). Oversigt over de danske Elateride. Larver. *Entomol. Meddelelser*. Kjöbenhavn.

- (6) HORST, A. (1922). Zur Kenntnis der Biologie und Morphologie einiger Elateriden und ihrer Larven. (Insbesondere Untersuchungen über *Agriotes obscurus* Linn.) *Arch. Natg.* Berlin, Abt. A, LXXXVIII, Heft 1, 1-90 (3 pls. and 102 text-figs.).
- (7) IMMS, A. D. (1924). *A General Textbook of Entomology*, pp. 64-96. London.
- (8) ROBERTS, A. W. R. On the life history of "wireworms" of the genus *Agriotes*, Esch., with some notes on that of *Athous haemorrhoidalis*, F. Part II. *Ann. App. Biol.* VIII, 193-215 (1 pl. and 4 text-figs.).
- (9) SCHIÖTTE, J. C. (1870). De metamorphosi Eleutheratorum observationes. (Bidrag til Insekternes udviklings historie.) *Nat. Tids.* ser. 3, VI, 467-536 (pl. I, figs. 1-15, pl. II, figs. 1-22).

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## THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

### 1. THE RELATION BETWEEN DEGREE OF INFESTATION AND WATER SUPPLIED

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(With 3 Text-figures.)

AN opinion prevails among many entomologists who have studied outbreaks of Thysanoptera in various parts of the world, that epidemic damage from certain species is connected in some way with abnormal meteorological conditions, and in particular with sub-normal rainfall.

It must be emphasised that this opinion rests more upon observation than upon precise experiment, and so expresses rather the point of view of the particular observer than any concrete phenomenon. The views of different observers concerning the same insect are thus sometimes contradictory.

With regard to *Heliothrips rubrocinctus* Giard., the Cacao Thrips, a serious pest of cacao, mango, avocado and other plants in the West Indies, Surinam, Brazil, San Thomé, Gold Coast Colony, Florida and elsewhere; decrease during the rainy season seems due to conditions unfavourable to multiplication; the greatest abundance is in times of drought (Reyne, A., 1921, Surinam). Weather conditions seem to have a very considerable influence upon the abundance of thrips; if, during the normal dry season there are frequent showers, thrips are likely to be more numerous; the reason for this is not definitely known (Ballou, H. A., 1919, Grenada). New shoots on cacao appear at the beginning of the rainy season, and it is then that thrips begin to multiply on the cacao trees, the maximum appearance being just after the heavy rains of May and June; a certain degree of moisture seems to be necessary for the development of the thrips; at the height of the dry season they are at their lowest ebb, although the damage done by them may be most apparent at that time; thrips are naturally controlled by heavy showers of rain and by excessive drought (Urich, F. W., 1916, Grenada).

On one estate, two periods of abundance of thrips occurred during the year, one in April and May, in the dry season, and the other in September and October, in the wet season. (*Agric. News*, Barbadoes, 1916, re St Vincent.) *Heliothrips rubrocinctus* seriously attacked and defoliated cacao especially in dry localities or during dry weather (Ritchie, A. H., 1916, Jamaica).

With regard to *Thrips tabaci* Lind, the Onion Thrips, a cosmopolitan pest of many cultivated plants, the periodicity of epidemics is in direct relation to the temperature and rainfall during June, July and August; a temperature above normal, and rainfall below normal, are factors that favour development and are unfavourable to the proper growth of the onion plant (Horsfall, J. L. and Fenton, F. A., 1922, Iowa). As a rule, when onions are planted, the dry season is not completely established and the first onion crop, lasting about two months, suffers little from thrips if sufficient water be present; there may be a second good crop, unless water be scarce, in which case, as in that of a third crop, a considerable loss occurs owing to infestation (Van Heurn, W. C., 1923, Java).

With regard to *Euthrips nicotianae* Hinds., the Tobacco Thrips, it is quite evident that the amount of injury by thrips will vary from year to year, depending upon the period and amount of rainfall (Hooker, W. A., 1907, Florida and Georgia).

With regard to *Scirtothrips citri* Moul, the Citrus Thrips of California, the number of generations is greater in exceptionally long, hot summers than in more moderate ones. Cool, cloudy or rainy weather markedly diminishes the various activities of the insects (Horton, 1918, California).

With regard to *Heliothrips indicus* Bagn., the Cotton Thrips, during the rainy season the thrips is difficult to find and only becomes effective after the rains have more or less ceased (Bedford, H. W., 1921, Sudan).

Many more quotations concerning this question could be given, but these will suffice.

Application of the belief that thrip epidemics are correlated with unusually dry conditions, to the practical control of an epidemic, has only been suggested, to the author's knowledge, by Hooker, who indicated in 1907 the noticeable effect of sprinkler irrigation in decreasing the numbers of *Euthrips nicotianae* upon tobacco plants, and the inability of surface irrigation to produce such a decrease; and by Corbett (1919) and Bedford (1921), who have recommended, against *Heliothrips indicus* in the Gezira cotton growing area of the Sudan, an increase in

the water supply of furrow irrigated cotton from 450 cm. per feddan (= 1.038 acres) to 550 or even 600 cm., every 15 days.

### **Scope of the present experiments.**

The series of experiments which are described in this paper were devised in order to decide:

(a) whether the degree, to which a species of Thysanoptera may infest a species of plant, can vary with the amount of water the plant receives;

(b) whether, if such correlation be established, there is any difference between rainfall and irrigation in this respect;

(c) whether there is any correlation between the degree of infestation and the interval of time which elapses between successive waterings of the plant.

The experiments were carried out under glasshouse conditions in Manchester during, as regards preliminary experiments, the summers of 1924 and 1925, and as regards the main experiments, the summer and autumn of 1926. About 300 cotton plants were used each year, chiefly of the Egyptian Sakellarides variety, and long staple American Upland varieties.

The species of thrips used in the experiments was *Thrips tabaci* Lind., which is in very many respects one of the most suitable species for such experiments. The biology and life-cycle of this insect will be discussed in later papers. It is sufficient here to indicate that this species possesses the following suitable qualifications.

(1) It is cosmopolitan, a severe pest of many plants in such climatically different areas as South Europe, West and Central Europe, Asiatic Russia, India, Java, Australia, Hawaii, Chili, Mexico, the United States, and Canada.

(2) Although not itself a recorded pest of cotton, it attacks cotton plants with avidity, producing lesions almost indistinguishable from those produced by *Heliothrips indicus*, the cotton thrips of the Sudan, and by *Thrips flavus*, the cotton thrips of Turkestan. It is the most closely related species to the latter form.

(3) It is particularly a species which appears in the form of sporadic and severe epidemics; such outbreaks have been recorded in areas so separated geographically as Iowa, France, Java and Queensland, and have been correlated by observers with drought conditions.

### Objections to glasshouse experiments.

There are of course certain serious objections that can be raised against experiments of this nature carried out under glasshouse conditions. In the first place, it might appear that the blocks of plants are too small numerically to permit of definite conclusions being drawn from the data thus obtained, and, in the second place, that the degree of insect infestation is higher than occurs normally in the field, since the plants are living under abnormal conditions of water supply, atmospheric humidity, crowding, root freedom, and so forth, whilst the insects concerned are protected from enemies and from adverse climatic conditions. Whilst admitting that glasshouse experiments are valuable only as a guide to the lines upon which large scale field experiments can profitably be conducted, and that field tests must be the final arbiters of the conclusions drawn from such glasshouse experiments, it must be emphasised that they permit of more precise control of temperature and water supply, and of a much more accurate conception of the degree of insect infestation, than can be obtained under field conditions. The plants, themselves, though from the same strain of seed, were finer and healthier plants vegetatively than were those in the Egyptian or Sudanese fields, owing possibly to the calmer and more humid atmosphere, and the lower sun temperatures, of the glasshouse; and although they were inferior in production of flowers and bolls, would be little inferior in the number of ripe bolls produced, were the glasshouses situated in a sunnier situation than the confines of an industrial city, since the degree of boll shedding was lower than in their native area.

As regards the degree of insect infection, it was probably higher under glasshouse conditions than in the case of ordinary endemic attack in the field, but probably much below the severity of infestation during an epidemic.

### Conditions of temperature and humidity.

With regard to the mean values for temperature and atmospheric humidity in a glasshouse, it is evident that whereas temperature is, within limits, controllable, the humidity is not thus controllable, and when considerably heavy amounts of water are being applied to plants, the mean relative humidity will approach saturation.

It was necessary therefore to carry out a series of experiments with the object of establishing:

(a) the optimum temperature for the development of the life-cycle stages of *Thrips tabaci* in a saturated atmosphere;



(b) the water supply required by cotton plants grown at this particular temperature and at a high degree of relative humidity.

The experiments concerning the optimum temperature for the insect will be described in a later paper. It may be stated briefly that the value arrived at was 20–22° C.

The value of water supply arrived at, for Egyptian and American plants grown in pots of 22–23 cm. diameter at the soil surface, in a mean temperature of 20–22° C. and at degrees of humidity between 80 and 100, was 300 c.c. of water per pot at intervals of three days. This is equivalent to 75 mm. of rainfall, or approximately 300 cm. of irrigation water per acre, per month. Throughout the experimental work of 1926, therefore, the temperature of the glasshouse was controlled, as far as possible, so as to give a mean value of 21° C. Actually, owing to fuel shortage, the mean temperature attained was 19° C., the mean maximum being 26·7° C. and the mean minimum being 11·25° C.

The mean maximum and minimum relative humidities throughout the period were 92·4 per cent. and 74·7 per cent. respectively.

### **The estimation of intensity of infestation.**

In order to be in a position to compare the intensity of thrip infestation of different blocks of plants throughout the period, a sufficiently accurate method of measuring the degree of infestation had to be devised.

There is no standardised method of estimating the density of insect attack that can be satisfactorily applied to foliage insects in general. Three lines of approaching the problem were possible:

1. Estimation of the *physiological* effect of insect attack upon the plant, by comparison of the amount of leaf shedding, flower production, square shedding and boll shedding of infested plants and uninfested plants.
2. Estimation of the *morphological* effect of insect attack upon the plant by obtaining the ratio of damaged to undamaged leaves.
3. Estimation of the *actual numbers* of insects upon the plant by counting.

The first method was impracticable, since many factors beside insect attack can influence leaf shedding or boll shedding or flower production.

The second method was also unsatisfactory since extensive leaf damage is no indication of correspondingly severe thrip infestation. Severely damaged leaves are generally free from thrips. Further a lightly attacked leaf may in time develop intensely rusty, necrosed areas.

The third method is impracticable, if precise accuracy be required, since, ideally, every stage of the insect on each plant of each block would have to be counted, and the minute size of these stages, together with the large number of them that may occur on a plant, would render the method inexpressibly tedious and slow. Even if only a certain number of leaves be examined, as was done by Reyne, there is the disadvantage that since leaves vary in size, such a count is useless for comparison with other blocks or with blocks of another species of plant.

The method actually adopted was to take, at intervals throughout the summer, a number of leaves from each block of plants, to measure the area of each leaf with a planimeter, to count the number of adult and larval thrips on each leaf, and to obtain for the sample of leaves a definite numerical *infestation factor* which expressed the number of adults or larvae per 100 sq. cm. of leaf surface. This factor was considered to represent the intensity of infection of that particular block of plants at that particular time.

The leaves were selected at random, but very young leaves and severely damaged leaves were avoided, since they were almost invariably free from thrips. Estimation of the intensity of infestation of each block was commenced only after the block had been subjected to a definite water supply for at least four weeks.

The method is of course not precise. Errors occur in measuring the area of each leaf. Errors in counting the number of thrips per leaf, since, when several hundred stages are present, even though the count be made with the help of a binocular microscope, small and partly concealed individuals are liable to become overlooked. The method however is sufficiently accurate to indicate differences in the degree of infestation of different blocks of plants, and although each leaf requires an examination lasting three to five minutes, the operation is incomparably quicker and less tedious than would be an attempt to count the insects on leaves still on the plant.

The method has the advantage of permitting comparison between blocks of plants of different ages and sizes, between plants of different varieties, and between plants of different species.

The value of the data thus obtained is dependent upon:

- (a) the number of plants examined;
- (b) the frequency of examination;
- (c) the purity of infection;
- (d) the non-migration of thrip stages between adjacent blocks.

Not more than one or two leaves per plant can be removed at each examination if the plant is not to be affected by defoliation. Actually only 10-20 leaves per block were taken at each examination, although each block contained 30-50 plants. Each block was examined at least four times within a month, that is to say within the average length of the thrip life-cycle.

The purity of infection throughout the experiments was very high. Occasional specimens of *Parthenothrips dracaenae* that occurred in early summer were easily noted and avoided when counting. No other species of Thysanoptera were present. There was no other cotton attacking pest such as Greenhouse Whitefly or Red Spider, beyond a slight infection of a few plants of one block by the aphid *Myzus persicae* during August 1926, and a slight attack by an unidentified Tortricid caterpillar during the same period.

The migratory powers of *Thrips tabaci* depend very largely upon the agency of wind. In an absolutely calm atmosphere, the insect can only leap from one leaf to another in close proximity, or, in the case of small plants, can travel from one plant to another by leaping along the ground. In a glasshouse, air currents are present to a slight extent, and a certain amount of migration of adult thrips occurs between blocks. It must be noted however that the thrips, after emerging from the pupal stage, climbs the nearest plant, works its way upward from leaf to leaf until it has found a lower leaf surface sufficiently clear to its liking, whereupon it commences to feed and to oviposit. Such oviposition is invariably upon the lower surface, and the insect probably only travels to the upper leaf surface after oviposition is over. That is to say, migrating thrips have in most cases already oviposited on the plant near whose base they emerged. In any case, there is no migration of larval stages from block to block.

Under glasshouse conditions therefore, the infestation of a series of adjacent blocks of plants can be compared throughout a season with a fair degree of accuracy, since the infection of each block is uniform, and a block is not reinforced greatly in number of adult thrips from an adjacent block, and in larval thrips not at all, providing that a sufficient interval between blocks be maintained.

In the field such comparison is difficult owing to the close proximity of plants and to the occurrence of wind. Even in one block, under field conditions, the windward edge may be heavily infested and the leeward edge relatively free.

In calculating the infestation factor, it is advisable to examine both

surfaces of the leaf and to double the value of each measured leaf surface since, although in the great majority of cases, the insects occur only on the lower surface, examples occur of both surfaces being infested by adults or larvae, owing to overcrowding or to severe surface damage of the lower foliage surface.

The highest value obtained for the infestation factor was, in the case of adults 27, and in the case of larvae 111 individuals per 100 sq. cm. of leaf surface. Both values occurred on a particular block of American cotton during late July and early August. The largest number of adults on an individual leaf was 84, and of larvae 392, again on American cotton. Counts of round about 200 larvae per leaf were common.

Four series of experiments were carried out.

### Series I.

Three blocks of plants, *E*, *F*, and *G* were taken. Blocks *F* and *G* were plants grown from severely selected Sakellarides seed of the 1924 Egyptian crop. Block *E* was from seed of the Maarad variety, an Egyptian mutant from the Arizona strain of Pima cotton, itself a descendant of the Egyptian variety Abbassi, and very similar to Sakellarides. The plants in each block were 20 cm. apart, the blocks were 60 cm. apart. The plants were grown in pots of 23 cm. diameter at the soil surface, whose drainage holes had been plugged with cement. The soil was a medium clay loam.

These blocks were watered at intervals of five days. *E* received the equivalent of 150 mm. of rainfall, or approximately 600 cm. of water per acre, per month; *F* received half this amount; *G* received one-fifth of this amount. The water was applied to the soil surface, care being taken to avoid splashing the foliage. Block *E* thus received an excessive water supply; block *F* a sufficient water supply; block *G* a deficient water supply. This was reflected in the appearance of the plants, block *E* comprising tall plants with numerous large succulent leaves, block *F* having plants similar in appearance to those in the field, and block *G* being composed of stunted plants with scanty, leathery, small leaves, the plants resembling the volunteer plants which may occur on fallow, unirrigated areas of the cotton field.

Fig. 1 indicates the degree of infestation of both adult and larval thrips on these blocks throughout the months of August and September, when infestation was highest. As shown by these curves, the infestation factor varied inversely with the amount of water supplied throughout the period; the mean infestation factor for the adults was, for *E*, *F* and

*G*, 0.42, 2.26 and 6.26 respectively; for larvae it was 2.85, 6.37 and 10.8 respectively.

If however the actual number of thrip stages per leaf be considered, it may be noted that *E* had 0.6 adults and 3.5 larvae, *F* had 2.3 adults and 6.9 larvae, *G* had 2.3 adults and 5.8 larvae per leaf. That is to say, these experiments suggest that excessive water supply may be accom-

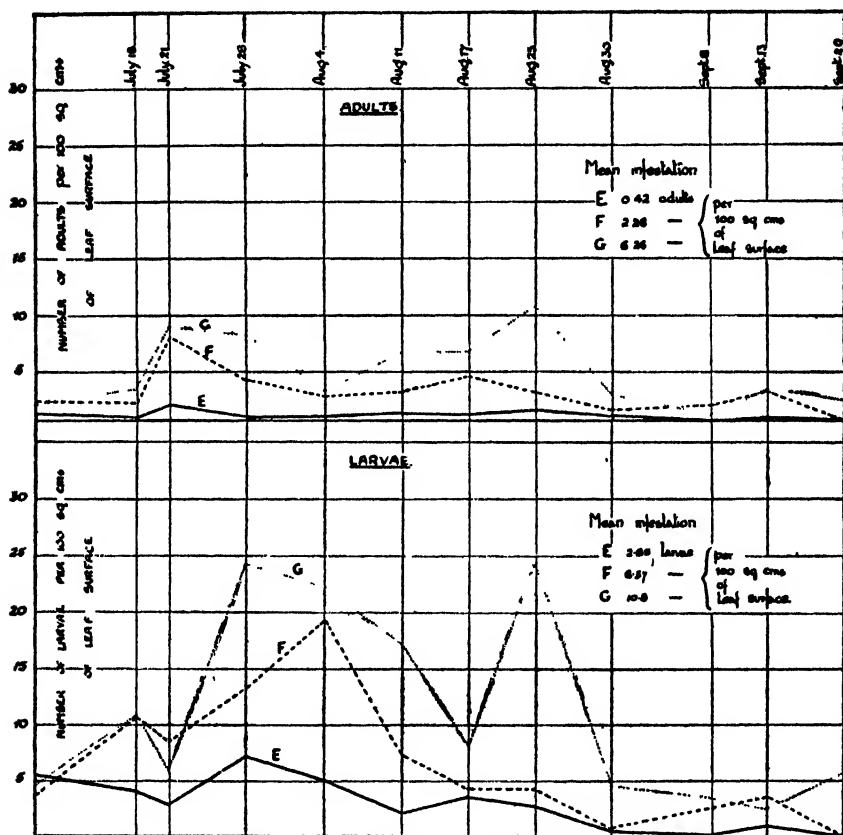


Fig. 1. The degree of infestation of three blocks of Egyptian cotton. *E*, *F*, *G*, receiving differing water supplies.

panied by a low infestation factor and by an actual low number of stages per leaf, as compared with plants receiving normal water supply.

Among plants suffering from water deficiency, the actual number of thrip stages per leaf may be lower than in the case of plants receiving normal watering, but owing to the smaller foliage surface the infestation factor will be greater and the plants will suffer more severely.

**Series II.**

Two blocks of cotton, *X* and *Za*, were taken, each comprising about 50 plants of a strain of Webber cotton, an American Upland long staple variety, from seed of the 1925 crop of the Zeidab estate of the Sudan Plantations Syndicate. The size and arrangement of pots was as before. Block *Za* was watered by a revolving sprinkler in a portion of the house that could be shut off from the rest by waterproof curtains. The height, speed and nozzle apertures of the sprinkler were so adjusted as to imitate fairly closely a heavy shower, and such a shower was applied at intervals varying from two to five days, the amount of rainfall at each application varying between 10 and 30 mm. but being always precisely recorded in a gauge. The rainfall intensity thus obtained was approximately 50 mm. per hour. The monthly rainfall simulated was that normally recorded in the Mongalla district of the southern Sudan, a district where long staple Upland cotton is being experimented with as a rain crop (Wardle, 1926).

Block *X*, on the other hand, was subjected to an irrigation system of watering, each plant receiving an amount of water equivalent in depth of millimetres to that which each of the *Za* plants was receiving, the watering of both blocks being carried out at the one time; that is to say, at the same time that the *Za* plants were receiving a sprinkler fall of, for example, 15 mm., the *X* plants were each receiving the equivalent of 15 mm. applied carefully to the soil surface of each pot.

The actual quantities of water applied each month were as follows:

	<i>May</i>	<i>June</i>	<i>July</i>	<i>August</i>	<i>September</i>
<i>Za</i>	130	110	120	160	90 mm.
<i>X</i>	5395	4565	4980	6640	3735 c.cm. per pot.

Thus the monthly totals were in each case in excess of normal requirements under glasshouse conditions.

Fig. 2 indicates the infestation factors throughout June, July, August and September. The curves show a marked difference between *X* and *Za* as regards the degree of infestation of both adults and larval thrips.

The mean infestation factors for block *X* for the whole period, were, for adults and larvae 6.58 and 16.8 respectively; for *Za*, the factors were 9.16 and 27.5 respectively.

The mean number of adults and larvae per leaf were, for block *X*, 7.2 and 17.1 respectively, and for block *Za*, 9.16 and 27.5 respectively. The close agreement of the actual numbers per leaf with the numbers

per 100 sq. cm. of leaf surface is due to the fact that if a large number of leaves of American plants be measured the total surface area of both sides of a leaf will be found to approximate to 100 sq. cm.

That is to say, the plants subject to irrigation were less infected on the whole than were those subject to rainfall, despite the fact that the

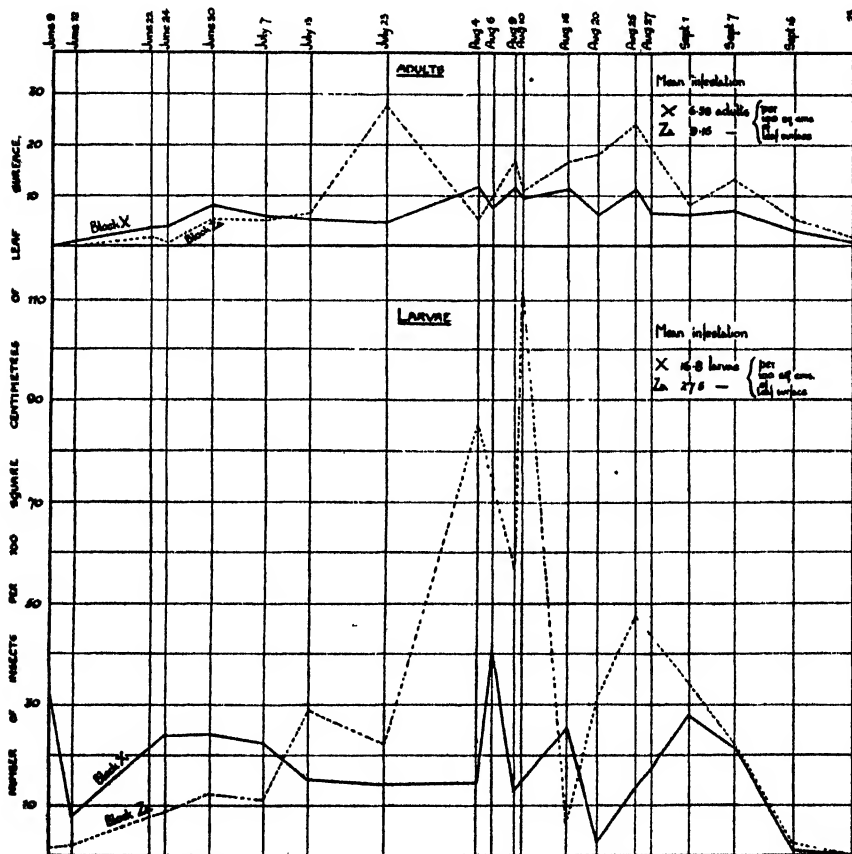


Fig. 2. The degrees of infestation of irrigated and of rainfall. American cotton with identical monthly water supplies.

amount of water supplied and the watering dates were identical. It may be noted however that up to July 10, block X was more infected than block Za, but after this date, as the plants became leafier and as, presumably, less water actually reached the soil level of Za than of X, the position became reversed.

These results oppose the view that the minimising effect of heavy

water supply is brought about solely by mechanical removal of thrip stages from the foliage. In the case of *Thrips tabaci*, both adults and larvae are negatively phototropic, quickly migrating under experimental conditions from a sun-illuminated side of a leaf to the shady side. On the plant, however, when the under surface of a leaf is excessively crowded, the hunger stimulus brings them to the upper surface, even when fully exposed to the sun; they are however very restless under such conditions. This migration to the upper surface is more marked when the leaf is shaded by others or when the sky is overcast. Adult thrips on the upper surface of a leaf are readily removed by spraying or by shaking the leaf, but larvae cling more tenaciously to the leaf.

The infestation factors for adults and larvae were obtained just before watering, and just after watering with the sprinkler on several occasions, and the results obtained suggest that the number of adults may be reduced considerably by the mechanical action of falling water, but that the reduction of larval numbers is slight. It is very possible however that many adults knocked from the plant succeed in getting back on to the plant again.

It is possible however that in a rainfall climate, a species of Thysanoptera, which lives habitually on the upper foliage surface, may be affected numerically by intense heavy periods of rain, or in a dry climate may be so affected by heavy and careless irrigation, but that some additional explanation for the influence of heavy water supply upon thrip infestation is necessary seems indicated by the greater influence of irrigation watering over sprinkler watering in the experiments.

### Series III.

This series comprised experiments devised to test the effect of water contact upon thrips stages. Batches of first-stage and second-stage larvae, of prepupae and pupae and of adults, were floated on the surface of water in closed vessels, and batches were placed in waterlogged soil in closed vessels.

Despite the fact that the insects were thus deprived of food, and were subjected to more prolonged and more intense contact with water than could occur under normal field conditions, the stages were very resistant.

No adults died within six hours, about 20 per cent. within 12 hours, 50 to 75 per cent. within 24 hours. All were dead within two days.

Of second stage larvae, 30 per cent. died within 24 hours, about 50 per cent. within two days, the rest within three days. First stage larvae



were less resistant, but the absence of food may have affected them. Prepupae and pupae were very resistant, very few dying within two days and many eventually becoming adults after several days' water contact. In a series of experiments conducted by my colleague Miss E. I. MacGill, second stage larval thrips were reared in glass chambers in which the relative humidity could be kept constant by the use of mixtures of sulphuric acid and water. The laboratory temperature fluctuated between 10.5 and 20.5° C.

At 100 degrees of relative humidity, 8.5 per cent. of larvae were reared through to the adult condition. At 20 degrees of humidity, 2.2 per cent. of larvae were reared through.

When prepupal stages were used at 100 per cent. of relative humidity, 40 per cent. of prepupae were reared to the adult condition; at 20 per cent., 37.5 per cent. were reared through; at absolute dryness 16.6 per cent. were reared through. Thus, although a large percentage of larvae and prepupae died before reaching the imaginal condition, such mortality seems due to the unnatural rearing conditions rather than to the influence of a high degree of humidity.

The results of this series of experiments therefore show that while certain stages of thrips can be killed by prolonged contact with water, they are not affected adversely by a saturated atmosphere.

#### **Series IV.**

Four blocks of plants were taken, *A*, *B*, *C* and *D* respectively, all comprising plants from Sakellarides seed of the Egyptian 1924 crop. These blocks were grown in the ground in a stiff clay loam. In blocks *A* and *B* the plants were grown on ridges; in *C* and *D* they were grown on the flat soil surface.

These blocks received water applied to the ground surface precisely as under irrigation conditions; each block received the equivalent of 225 mm. of rainfall, or approximately 900 c.m. of water per acre, per month. The monthly total was applied in equal instalments at intervals varying for each block; thus *A* received the equivalent of 90 mm. every 12 days, *B* the equivalent of 60 mm. every 8 days, *C* the equivalent of 30 mm. every 4 days, and *D* the equivalent of 15 mm. every 2 days. A water supply of 900 c.m. per acre per month would not be excessive under the conditions of low humidity, high sun temperature, and high rate of surface evaporation in such an area as Arizona, Mesopotamia or the Sudan, but under glasshouse conditions it is grossly excessive, being in this case three times what is necessary. In the case of block *D* the

soil was perpetually under water. All the plants grew excessively tall and strong, and had huge leaves and few flowers.

The degree of thrip infestation throughout the period was low. The curves of the infestation factors are given in Fig. 3.

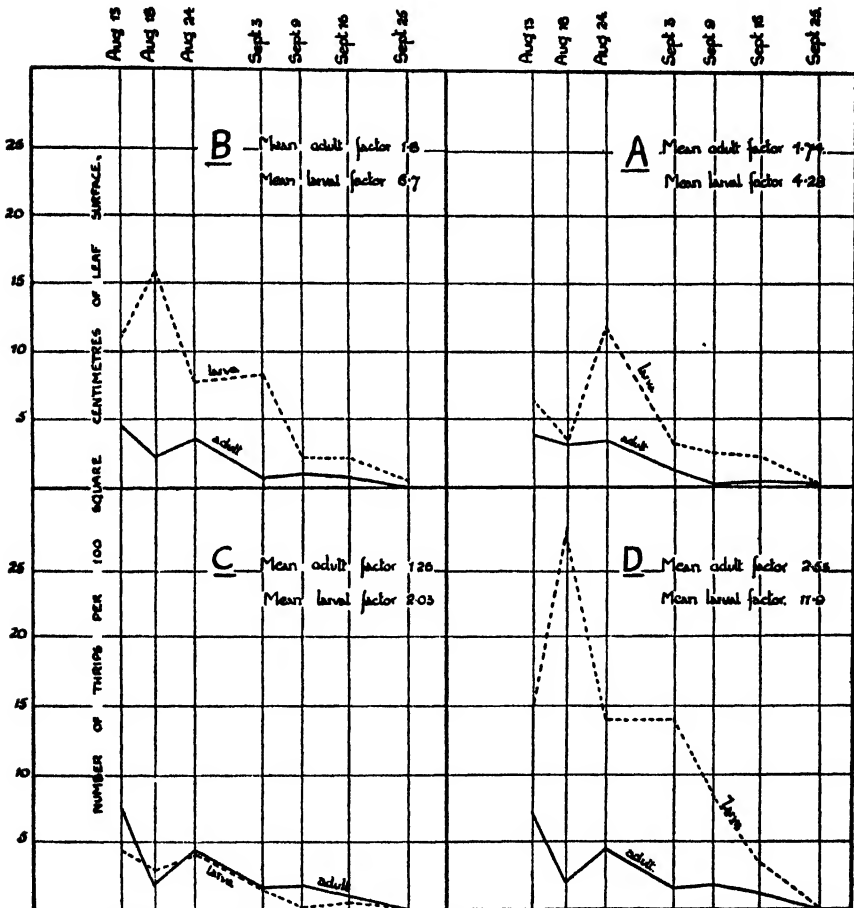


Fig. 3. The degrees of infestation of four blocks of Egyptian cotton receiving identical monthly water supply but at differing intervals.

It will be noted that block *C* was the least infested, *A* the next, then *B* and then *D*, the respective mean factors being, as regards adult infestation, 1.26, 1.74, 1.8 and 2.53; and as regards larval infestation, 2.03, 4.28, 6.7 and 11.9 respectively. The close similarity between the mean factors for adult infestation is probably due to the fact that,

owing to the excessive vegetative growth of these blocks, the branches of one block tended to overlap those of adjacent blocks and so to offer a path of migration for adult thrips from one block to another. An attempt to isolate the blocks by intervening hedges of *Cajanus indicus* was unsuccessful, as these plants, although in the Sudan apparently avoided by *Heliothrips indicus*, were attacked as freely as were cotton plants and thus merely aided migration of the adult thrips from one block to another. Such adult migration however seems to occur after oviposition, and there is no larval migration between adjacent blocks, so that the curves of larval infestations give a more correct conception of the discrepancy between the mean infestations of the different blocks. The results of this series of experiments suggest that mere weight of water is not the only factor in bringing about a reduction in the degree of thrip infestation, but that the nature of the ground surface or the intervals between watering probably play a part.

Block *C* had a flat ground surface, water was applied every four days, and the soil surface although moist was firm and caked between waterings; block *A* had a ridged surface, but owing to the amount of water applied at each watering these ridges were completely covered, and for a few days after watering were compact, but, before the next watering date, had become loose and powdery; block *B* had also a ridged surface, but the water was never deep enough at each watering to cover the crests of the ridges, which were therefore always somewhat loose; block *D* had a flat surface but was almost always under a few millimetres of water.

It may be added that since all the plants received the same quantity of water per month, all were about the same height and same stage of development. There is no reason to believe that the blocks differed from one another, as regards concentration of cell sap, thickness of cuticle, or other morphological features, so markedly as did the degrees of larval infestation upon them. As regards age, only 20 days separated the earliest sown block from the latest.

#### *General conclusions.*

It is probable that in very many cases the effect of heavy rainfall or of heavy irrigation in reducing thrip infestation is more apparent than real. Increase of water supply stimulates the growth of individual leaves and the production of new leaves, so that the numerical ratio of thrip stages to unit area of foliage surface is lowered, and the ratio of damaged to undamaged leaves is lowered. The plants will therefore

appear to suffer less from thrip damage, and may actually do so although no numerical decrease of thrips may have been brought about. Further, under field conditions, where the chief danger to the plant from thrip attack arises from the defoliation caused by premature and undue leaf shedding, any meteorological phenomena or soil conditions which increase such leaf shedding or which reduce it, will magnify or will minimise, as the case may be, the damage resulting from thrip attack. That is to say, it is conceivable that of two adjacent districts, each with identical degrees of thrip infestation, but differing from one another as regards soil conditions and exposure to wind, the plants of one district may show severe injury, those of the other district may be scarcely affected. The results of these experiments however support the view that a correlation exists between the degree of infestation of *Thrips tabaci* upon cotton plants, and the amount of water the plants receive. They do not bear out the idea that the primary effect of watering is due to mechanical removal of thrip stages from foliage, or that the insects are readily killed by contact with water or waterlogged soil or inimically affected by a saturated atmosphere.

Further, there is no evidence that changes in the concentration of cell sap, induced in cotton plants of the same age by differing water duties, are sufficiently important to influence the degree of thrip infestation, although the migration of thrips from cotton in the field to such plants as lucerne (*Medicago sativa*) when the cotton is six or seven months old may be so influenced.

One reason for the influence of heavy rainfall or heavy irrigation watering upon the degree of infestation of certain thrip species probably lies in the phenomenon of surface caking to which certain types of soil are liable, when subjected to alternate periods of beating rain or heavy flooding, and of hot sunshine. In the case of those species of Thysanoptera whose larvae habitually descend into the surface layers of the soil to pupate, and whose emerging imagines must pass through the soil surface, such soil crusting, extending as it may do to a depth of several inches, may be a potent agent in limiting their numbers.

Towards the end of the summer of 1926, a number of pots containing Webber plants were selected at random, the plant in each cut down to within an inch from the surface. In the case of half the pots, the soil surface was kept dry and allowed to become loose and powdery; in the other pots, the surface was moistened and tamped down at intervals of a few days. From the dry powdery surface, 175 adult thrips emerged between September 9th and 20th. From the moistened crusted surfaces

only 49 thrips emerged during the same period. Further experiments were vitiated by the general disappearance of thrips from the cotton about this time, and more evidence is required, but the results obtained certainly suggest that the emergence of adults is influenced by the nature of the soil surface. The entrance of larvae into the soil to pupate is probably less affected by soil caking, since they can pupate away from soil. If the soil be completely under water, some larvae will pupate between fallen leaves or on the plant itself.

The effect of surface caking upon the thrip stages is probably one of suffocation brought about by the pressure of the clay particles.

The degree to which such surface caking occurs under the influence of rainfall or irrigation water, varies according to several factors. In soils with a low clay content, less than 15 per cent., such caking does not usually occur, the surface remaining loose and friable. With a clay content between 15 and 30 per cent., moderate showers tend to compact the soil, under temperate climatic conditions, and it will remain so compacted for a period of days. When the clay percentage is high, more violent water pressure is necessary, in the form of heavier rain showers or heavier irrigation waterings, and the duration of the crusted condition is shorter. Under conditions of high sun temperature, low humidity and consequently high surface evaporation, soils of high clay content do not remain compacted long but tend to dry rapidly, to shrink greatly, and to become fissured with large cracks.

The tendency of clay loams of humid, high rainfall areas to undergo surface caking may be of course reduced considerably by tillage, by the presence of calcium carbonate or calcium sulphate or humus in the soil, and by surface vegetation, so that, on the whole such areas afford favourable conditions for the endemic presence of soil pupating Thysanoptera and for epidemic attack during periods of long intervals between rainfall showers.

On the other hand, in arid or semi-arid areas, the tendency of the heavy clay, calcareous soils, to show surface pulverulence and so afford favourable thrip conditions, is offset by the almost invariable presence in such soils of sodium or potassium carbonate which, even in low percentage, favours soil compaction. Further, the native flora tends towards thickness of epidermis and so is antagonistic to thrip attack. That is to say, such areas are not favourable to the multiplication of Thysanoptera.

Scientific large scale cultivation of arid or semi-arid soils, such as occurs to-day in the Sudan, the south-west United States, Mesopotamia,

north-west India, and elsewhere, is a comparatively modern practice. Improved methods of tillage, of drainage, of deep furrow irrigation, of gypsum addition, and so forth, can neutralise the action of alkaline salts to some extent, and can enable the production of crops which under primitive conditions of agriculture would not tolerate such soils, such crops for example as long staple varieties of cotton, cruciferous crops, leguminous crops such as alfalfa (*Medicago sativa*), the latter plant a notoriously favourite food plant of thrip species in many parts of the world. That is to say, modern agricultural conditions tend to make semi-arid areas favourable for Thysanoptera, and if an adaptable species should become introduced into such an area, the tendency would be for it to become well established, to become endemic as regards damage, and severely so. The weight of water supply necessitated in such areas, owing to the high degree of surface evaporation, does of course tend to promote surface caking and so possibly to oppose thrip multiplication, but the value of this is offset by the long intervals between waterings, by tillage and by furrow irrigation in which the crests of the ridges are untouched by water and hence remain powdery. Such agricultural practices however are unavoidable in the case of the cotton plant, since the effect upon yield, induced by the lower degree and later appearance of flowering, and by lower boll production, which more frequent watering or tillage neglect would produce, would outweigh any possible advantages resultant from thrip production.

In conclusion, I must acknowledge my great indebtedness, for research facilities and for information, to the following: the Empire Cotton Growing Corporation, the Sudan Plantations Syndicate, Mr H. King, Mr H. W. Bedford and Mr Johnston of the Sudan Government Entomological Service, Mr Walley of the Gezira Research Farm, Mr C. B. Williams of the Egyptian Entomological Service, Mr Nichol of the Arizona Entomological Service, the staff of the Botanical Department of this University, Mr H. Britten of the Manchester Museum.

#### SUMMARY.

1. Experiments concerning the degree of infestation of *Thrips tabaci* upon cotton plants under glasshouse conditions suggest that the *infestation factor*, or number of thrip stages per 100 sq. cm. of foliage surface, varies inversely with the weight of water supplied.

2. Plants receiving excessive water supply have a lower infestation factor and a lower number of thrip stages per leaf as compared with plants receiving sufficiency of water supply; plants receiving insufficient water supply have a greater infestation factor than plants receiving sufficiency of water supply but have not necessarily a greater number of thrip stages per leaf; owing to their smaller foliage area however they suffer more from thrip attack.

3. Irrigation is more effective in influencing the degree of thrip infestation than is rainfall.

4. Irrigation on a flat soil surface is more effective than irrigation on a furrowed soil surface.

5. The effect of excessive water supply does not appear to be due to mechanical removal of thrips stages from the foliage, nor an injurious effect of soil moisture or atmospheric moisture upon thrips stages, nor alteration of the osmotic concentration of cell sap, although all three factors may operate slightly.

6. It is suggested that one factor concerned is the influence of heavy water supply upon the texture of certain soils, in promoting surface caking which will act inimically to soil pupating species of Thysanoptera.

#### REFERENCES.

- (1) BALLOU, H. A. (1919). Report on Cacao Thrips in Grenada. *Report to Governor of Windward Islands, Grenada.*
- (2) BEDFORD, H. W. (1921). The Cotton Thrips (*Heliothrips indicus*. Bagn.) in the Sudan. *Bull. 18, Ent. Sect. Wellcome Trop. Res. Lab. Khartoum.*
- (3) CORBETT, G. H. (1920). Observations on the Cotton Thrips in the Gezira, Blue Nile Province, Sudan, in 1918-19. *Bull. Ent. Res. London, xi.*
- (4) HOOKER, W. A. (1907). The Tobacco Thrips. *Bull. 65, U.S. Dept. Agric.*
- (5) HORTON, J. R. (1918). The Citrus Thrips. *Bull. 616, U.S. Dept. Agric.*
- (6) HORSFALL, J. L. and FENTON, F. A. (1922). Onion Thrips in Iowa. *Bull. 205, Iowa Agric. Exp. Sta. Amesbury.*
- (7) REYNE, A. (1921). De Cacaothrips. (*Heliothrips rubrocinctus* Giard.) *Bull. 44, Dept. v. d. Landbouw, Suriname.*
- (8) RITCHIE, A. H. (1916). Report on Entomologist for year 1915-16. *Rept. Jamaica Dept. Agric.*
- (9) URICH, F. W. (1916). Insect Pests in Trinidad. *Entomologists' Report. Minutes Meeting Bd. Agric. Trinidad.*
- (10) VAN HEURN, W. C. (1923). Over een waarschijnlijke parasiet (*Thripoctenu brui* Vuell.) en een vermoedelijke hyperparasiet van *Thrips tabaci* Lind.
- (11) WARDLE, R. A. (1926). *Tijdschr. Ent. lvi.*
- (12) — (1926). The climate of the Southern Sudan in relation to cotton growing. *Mem. and Proc. Manchester Lit. and Phil. Soc.*

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## THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

### 2. THE RELATION BETWEEN TEMPERATURE AND LIFE CYCLE IN A SATURATED ATMOSPHERE

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(With 3 Text-figures.)

As indicated already in the first part of this paper, a correlation between the degree of infestation of a plant species by a thrip species, and the weight of water supply that the plant receives, is suggested by the results of experiments with *Thrips tabaci* Lind. infesting cotton plants under glasshouse conditions.

Under such conditions, the mean relative humidity throughout the experiments cannot be controlled so readily as can the mean temperature, and where water supply experiments are being carried out, the mean relative humidity may approach saturation. In this respect, of course, the environment of plant and insect will resemble that which prevails in the field immediately after heavy rainfall or heavy irrigation. It is therefore of some interest and importance to know the extent to which the metabolic processes of the insect are influenced by such high conditions of relative humidity, and particularly to establish the temperatures at which metabolism will be at its optimum, in so far as is indicated by the duration of life cycle stages, and by the percentage of transformations from one stage to the next.

The data put forward here is derived from experiments carried out under glasshouse conditions arranged, so far as possible, to simulate field conditions, and under laboratory conditions of constant temperatures and constant relative humidity percentages.

Of the laboratory data, however, only those obtained at a relative humidity of 100 per cent. will be discussed here.

The suitability of *Thrips tabaci* for these experiments has already been indicated. It may be stated briefly that this species is one of the most cosmopolitan species of Thysanoptera, having been recorded from Albania, Austria, Australia, Bermuda, Bessarabia, Bohemia, Dalmatia, England, Florida, France, Germany, Hawaii, Heligoland, Hungary, India, Italy, Java, North America, Russia, Sweden.



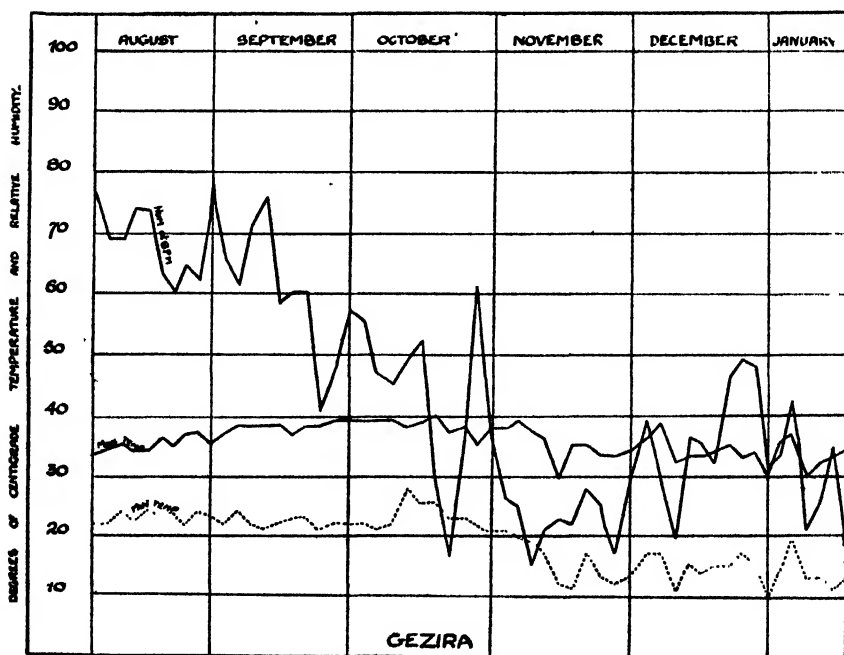
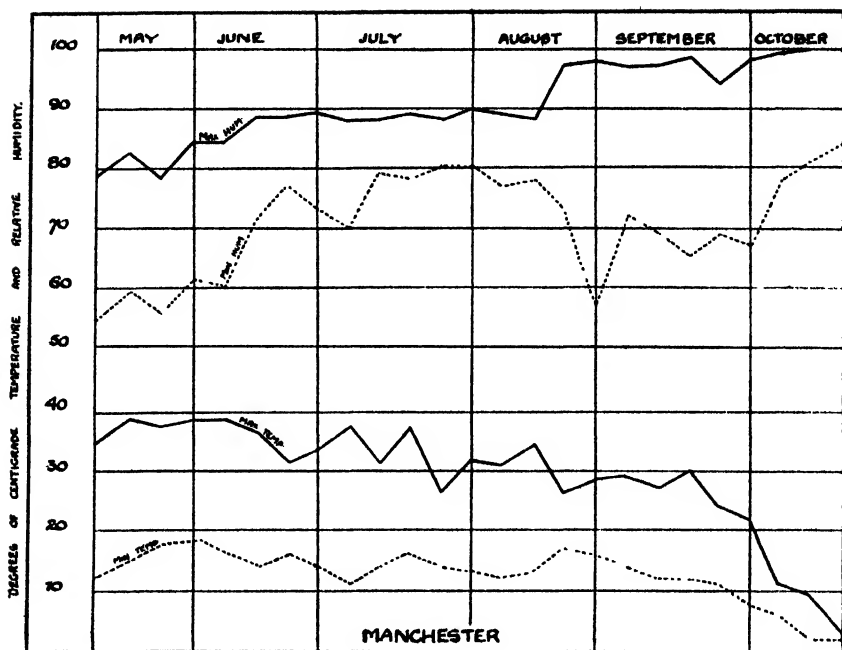


Fig. 1. Mean temperatures and humidities for Manchester (glasshouse) and the Sudan (Gezira).

That is to say, it is a species whose geographical range is not narrowly limited by climatic temperatures. It is further one of the most omnivorous of Thysanoptera, having been recorded from a very wide range of unrelated plants. It attacks cotton plants with avidity, producing lesions almost indistinguishable from those caused by the closely related *Thrips flavus* of Turkestan, and from those caused by *Heliothrips indicus* in the Sudan. Finally, it is particularly a species which occurs in the form of sporadic and severe epidemics.

In the glasshouse, the daily temperatures and relative humidities were recorded during the period May to October on a thermo-hydrograph. The mean weekly values are indicated in Fig. 1, and, for comparison, there are given the weekly mean temperatures and relative humidities for the Gezira district of the Sudan—an area where *Heliothrips indicus* is severely endemic upon cotton—for the period August to January, during which the thrips is active. As comparison between the respective curves will show, the chief difference between the two stations relates to humidity. Whereas the glasshouse humidity did not vary greatly around a mean of 84.5 per cent., the Gezira mean humidity fluctuates from a high value at the beginning of the season to a low value towards the end of the season.

On the other hand, the curve of Gezira temperatures is much more uniform than that of the glasshouse temperatures.

The appearance and disappearance of *Thrips tabaci* in the glasshouse are dependent upon temperature conditions. The appearance of *Heliothrips indicus* in cotton in the Sudan would seem, according to Bedford (1921) to be influenced by humidity conditions, the insect remaining on the cotton plant only so long as the relative humidity is low.

#### *The life cycle stages.*

As is usually the case in Terebrantian thrip species, the life cycle comprises egg stage, first stage larva, second stage larva, prepupa, pupa and imago.

The morphology of these stages in the case of *Thrips tabaci* has been fully dealt with by Van Eecke (1922) and need not therefore be discussed here.

The following points however deserve to be briefly noted. The first point concerns the variability in colour of the adults and larvae. Second stage larvae vary in colour from milk white to orange yellow. Adults vary from light yellow to brownish black. These colour variations occur irrespective of the mean temperature or the mean humidity at which rearing is carried out, and there can be little doubt that they are not

associated with differences of temperature and humidity. In the case of the genus *Heliothrips* whose species habitually, in the adult stage, roam about on the surface of foliage exposed to tropical sunlight, the adult colouration is usually brown or black, but in the case of *Thrips tabaci*, although at times adults occur numerously on the upper surface of cotton leaves, careful observation did not support the possibility that dark coloured individuals tend to frequent the upper surface of leaves more than light coloured ones do.

The second point concerns the proportion of male to female individuals. The male of *Thrips tabaci* has been described by Hinds (1903) and by Van Eecke (1922) and is stated to be similar morphologically to the female, but smaller in size. In the present case, nearly 3000 individuals of the adult stage, collected at intervals throughout the summer of 1926, were examined without a single male individual being found. Possibly males are produced towards autumn, but reproduction throughout the summer must have been parthenogenetic.

#### *Life cycle in the field.*

The ovaries of *Thrips tabaci* consist of four tubules on each side; the tubules are short and panoistic.

The mature egg is bean shaped and is approximately  $200\mu$  in length by  $100\mu$  in breadth. The immature egg is small and the large nucleus is clearly visible, but as the egg matures it becomes heavily laden with yolk which obscures the internal structure. The yolk appears to consist of a mass of globules and is clearly visible through the chorion.

The eggs are laid singly, scattered over the under surface of the leaf, but imbedded in the mesophyll tissue. The process of egg laying is a long one as the ovipositor is inserted deeply in the leaf tissue. In one case, a female took at least 15 min. to deposit a single egg, and this thrips was not observed until after the ovipositor had been inserted. The eggs can be distinguished under a binocular microscope as translucent spots, especially if a leaf is dehydrated and soaked in oil of cloves; if the embryo is well advanced, the egg can be detected as a small projection of the leaf surface. The newly laid egg does not differ greatly from the mature ovarian egg but is slightly larger; during embryonic development, however, the egg loses its bean-like shape and becomes oval; the embryo can be seen through the chorion and as the time of hatching approaches, the red larval eyes become conspicuous.

The number of eggs laid by one female is probably not great. Hinds (1903) gives the number in the case of *Anaphothrips* as being 50 to 60,

and states that from 30 to 40 per cent. of these hatch. In *Thrips tabaci*, the number of eggs laid by one female is possibly about the same as with *Anaphothrips*, since 10 to 20 larvae are obtainable from an individual female.

In the glasshouse, at a mean temperature of 19° C. and a mean relative humidity of 84·5 per cent., the duration of the egg stage between oviposition and hatching averages 8 days. The larva forces its way head first out of the egg and through the slit made in the leaf surface by the ovipositor of the adult. It is approximately 0·37 mm. long, the colour is translucent white, and head and appendages are very large in proportion to the rest of the body. The larvae are at first somewhat gregarious in habit, and feed clustered together, generally in the shelter of the somewhat protruberant leaf veins; as they become older and larger, they scatter over the leaf surface in search of fresh food, but do not seem capable of migrating from one leaf to another.

The age at which the first moult occurs seems to vary considerably, and the larva may be comparatively large before it reaches the second stage.

The second stage larva is approximately 0·9 mm. long, almost as large as the adult; the colour varies from pale to deep yellow; head and appendages are only a little larger than those of the newly hatched larva.

Under the conditions of temperature and humidity stated above, the two larval stages last, together, between 10 and 14 days.

Full-fed larvae as a rule leave the plant, but whether by migration down the stem or by simply falling from the leaf, is not definitely settled. They bury themselves in the surface soil, moult and become prepupae. Exceptionally, the prepupa is found on the leaf, sometimes in empty cocoons of a species of *Aphidius*.

The prepupa is similar in size to the second stage larva. It does not feed but is capable of movement, although not inclined to move from place to place unless disturbed. The duration of this stage is short, between 1 and 2 days in this case.

The pupa is similar in habits to the prepupa but its duration is longer, lasting between 4 and 7 days.

Thus the life-cycle between oviposition and imaginal emergence under the glasshouse conditions described is 23–31 days; later in the summer, with a mean temperature of 15° C., the life-cycle is at least 35 days.

The number of generations between May and October in the glasshouse was seven.

The observations of other workers concerning the life-cycle of *Thrips tabaci* may be tabulated as follows:

Locality	Observer	Temp. (° C.)	Egg stage (days)	1st and 2nd larval stages
Holland	Van Eecke	?	7	8
Massachusetts (glasshouse)	Hinds	?	4-7	7-8
Florida	Quaintance	?	3½-4	7-9
Iowa	Horsfall and Fenton	?	3-10	7-21
Manchester	MaoGill	19	8	10-14
(glasshouse)		15	8-11	16-19

Prepupa and pupa	Total
4 days	3-4 weeks
7 "	3-4 "
4 "	16 days
4-7 "	7 days ( <i>sic</i> ) to several weeks
1-2, 4-7 "	4 weeks
3-5, 8-12 "	5-6 weeks

Although no data as to mean temperatures and humidities have been given by other observers, it seems clear, from the variance of opinion concerning the duration of the several stages, that the total length of life-cycle of *Thrips tabaci* is prolonged or abbreviated by changes in temperature and humidity, and that this species is not an insect in whose life-cycle a curtailment or a prolongation of one stage can be compensated by a corresponding prolongation or curtailment of a succeeding stage, and so not an insect whose life-cycle remains constant as regards length throughout varying conditions of temperature and humidity.

*Life-cycle at constant temperatures, and constant humidity.*

Data concerning the duration of life-cycle stages in a constant saturated atmosphere and at constant temperatures, were obtained by using a Multiple Temperature Incubator, a simple type of that described by Williams (1924).

This consisted essentially (Fig. 2) of a copper hot-water chamber, 12 × 12 × 10 in. in dimensions, and a double walled zinc ice box of about the same size; the two boxes were connected by a 4 in. diameter tube of copper foil, nearly 6 ft. long, whose closed ends protruded each into one of the chambers. The temperature of the hot-water chamber was kept constantly at 63° C.; the ice box was replenished daily.

It must be noted that there was no passage of hot water along the tube, but by radiation of heat through the metal tube, a range of temperature, each constant within the limits of 1° C., was obtained along

the tube. In this case, the range of temperatures obtainable was from 7-50° C.

Along the tube was drilled a series of holes of  $1\frac{1}{2}$  in. diameter, at equal intervals, in each of which a corked tube was placed. The copper tube with its contained tubes was insulated by being wrapped round and round with alternate layers of cotton wadding and asbestos mill-board. The hot-water chamber was similarly insulated. Each tube contained enough distilled water to ensure a saturated atmosphere and was large enough to hold a piece of fresh cotton leaf for the support of thrip stages. The particular points of temperature made use of are indicated in the figure.

Fig. 3 indicates the percentage of larvae reaching the prepupal stage, the percentage of prepupae reaching the pupal stage, and the percentage of pupae reaching the imaginal stage, at a constant humidity value of

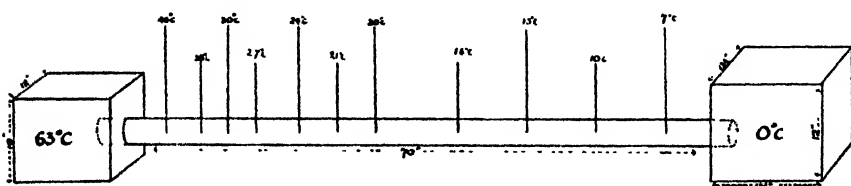


Fig. 2. Diagram to indicate dimensions and temperatures of the multiple temperature incubator.

100 per cent. of saturation, and at a number of temperature points between 7 and 47° C.

It will be noted from the curves that, in the case of larvae and pupae particularly, the temperature point at which the greatest percentage of individuals transformed into the next stage was 21° C. On either side of this temperature point, the percentages drop fairly regularly until, at each extreme of the scale, no adults hatched out.

At this temperature, 36.6 per cent. of larvae became prepupae, 95 per cent. of prepupae became pupae, and 91 per cent. of pupae became imagines. The great discrepancy between the number of larvae which failed to transform, and the numbers of prepupae and pupae which failed to do so, cannot be altogether attributed to loss during manipulation. Such loss did occur. The pieces of leaf in each tube had to be changed daily, and the minute stages had to be sought for and transferred with a fine brush. The 5 and 9 per cent. losses of prepupae and pupae are almost certainly wholly due to loss during manipulation. The reason for the fact that at temperatures of 7, 10, 13 and 16° C.,

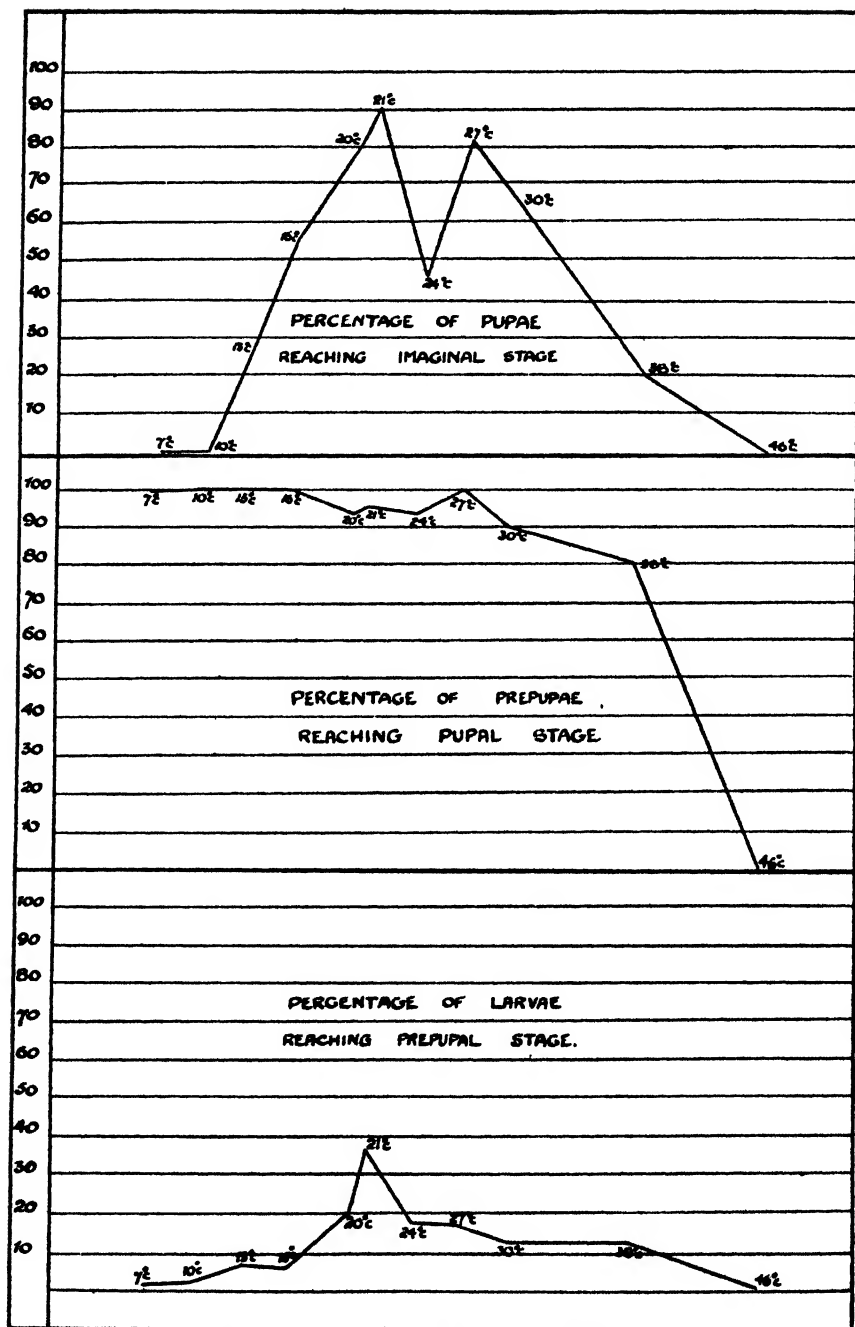


Fig. 3. The percentages of transformations from one stage to another at differing temperatures.

100 per cent. of prepupae became pupae supports this explanation, since at these temperatures so few pupae were obtained, that none were likely to be overlooked during transference.

The 66.6 per cent. loss of larvae cannot be attributed solely to loss during transference. Possibly the artificial conditions, absence of soil and so forth is the cause of so many larvae failing to transform. On the other hand, the occurrence of a high rate of larval mortality in the field seems indicated by the ratio of larvae to adults which occurred among the thousands of individuals counted during the series of leaf counts described in the previous part of this paper. This ratio was, for American cotton plants, 9 adults to 16 larvae; for Egyptian cotton plants it was 2 adults to 6 larvae; for *Cajanus indicus* it was 4 adults to 10 larvae.

Thus, in a saturated atmosphere, the percentage of larvae which go through to the imaginal stage is not high at any temperature point, but reaches its maximum at temperatures approximating to 21° C. At temperatures between 10 and 38° C., the percentage of prepupae and pupae which transform further is probably, excluding losses due to faulty experimental technique, absolute. The minimum effective temperature is below 7° C. and the maximum effective temperature is above 38° C.

The percentage of transformations at other degrees of relative humidity will be discussed in a later paper but such data as have already been obtained do not suggest that this percentage is higher at lower humidities than it is at saturation.

The larvae used in the incubator experiments were not very young, the majority being 3 to 4 days old, so that the duration values for larval stages, obtained by this method, are only approximate. At temperatures of 21° C. or higher, the maximum duration of a larval stage was 9 days, so that the total larval period was 12 to 13 days. At temperatures below 21° C., the maximum duration was 15 days, a total length of 18 or 19 days. The duration of prepupal and pupal stages increases greatly with fall of temperature. Thus:

Temperature ° C.	Average prepupal duration (days)	Average pupal duration (days)
38	1.44	2.2
30	1.22	2.3
27	2.0	4.4
24	2.5	4.0
21	2.0	3.7
20	2.1	4.8
16	3.1	8.0
13	5.1	12.6



It will be noted that temperatures below 21° C. appear to have a greater effect on the rate of development than do those above this.

At 38° C., the average prepupal duration is 1.44 days, and at 21° C., it is 2.0 days; that is to say, for a difference of 17 degrees of temperature there was only 0.56 of a day difference in the length of the stage. On the other hand, at 13° C., the average prepupal duration was 5.1 days, a difference from that at 21° C. of 3.1 days for a difference of 8° C.

Similarly, at 38° C., the pupal stage averaged 2.2 days, at 21° C., it averaged 3.7 days, at 13° C. it averaged 12.6 days. That is to say, there was a difference of only 1.5 days for 17 degrees of temperature difference above 21° C., and of nearly 9 days for the difference of 8–9° below 21° C.

That is to say, as with other biological processes, the rate of prepupal and pupal development of *Thrips tabaci* is greater at low temperatures than at high temperatures. Between the limits of minimum and maximum effective temperature, each degree rise of temperature is correlated with a shorter prepupal or pupal duration, but the quantity time by which the duration is shortened is not constant for each degree of temperature; it is a quantity which gradually diminishes as the temperature increases.

It may be pointed out that the work of Parker, J. R. on *Melanoplus atlantis*, as summarised by Chapman, R. N. (1926), suggests that the duration of an insect life-cycle stage, when subjected to a range of temperatures *varying* around a certain mean value, will be shorter than when subjected to constant exposure to that temperature value, so that life-cycle values obtained in laboratory experiments for constant temperatures values do not accurately represent life-cycle values at mean field temperatures.

Under laboratory conditions of constant temperature, there is considerable individual variation as regards the duration of life-cycle stages. Thus, at 38° C., although the majority of prepupal *Thrips tabaci* only lasted between 1 and 2 days, three individuals had a duration of 2 days, and two individuals had one of 3 days. At 27° C., though most of the prepupae transformed after a period of between 1 and 2 days, one abnormal insect had a prepupal stage of 8 days.

At 21° C., about one-half of the prepupae transformed between 2 and 3 days, but cases of a prepupal stage lasting 3 or 4 days were common. At 16° C., whereas the majority of prepupae lasted between 3 and 4 days, one individual pupated after only 1 day. At 13° C., the minimum duration of a prepupal stage was 2 days, and the

maximum was 10 days; the majority however went five or more days before pupation.

The pupal stage showed even more variation in length than the prepupal one. The shortest time on record was 1 day, and a few individuals with this very brief pupal stage were found at all temperatures between 27° and 38° C.; no pupal stage lasted a shorter time than 3 days at 24° C., but at 21° C. there was one 1 day pupa, and several 2 day pupae. The longest pupal stage at temperatures above 21° was 10 days at 27°, and it is interesting to note that the longest prepupal stage, in a different individual, for hyper-optimum temperatures, also occurred at this temperature. Below 21°, the length of the pupal stage increased rapidly with fall of temperature; at 20° there was one 2 day pupa, but the majority lasted 5 or 6 days; at 13°, a length of 17 days was recorded for one pupa.

Thus, although the rate of development in *Thrips tabaci* tends to increase with rise of temperature, there is considerable individual variation.

In the list of acknowledgments already given in the previous part of this paper, I would stress particularly the names of Mr H. Britten for help in the identification of various insect species, and Mr V. P. Walley, Chief Inspector of the Gezira Research Farm, Sudan, for information concerning meteorological data for that locality.

#### SUMMARY.

1. The life-cycle of *Thrips tabaci* from oviposition to imaginal emergence, on cotton plants, under conditions of temperature varying between a mean maximum of 26.7° C. and a mean minimum of 11.25° C., and of relative humidity varying between a mean maximum of 92.4 per cent. and a mean minimum of 74.7 per cent., has a duration of 23–31 days, comprising an egg stage of 8 days, two larval stages with a combined duration of 10 to 14 days, a prepupal stage of 1 to 2 days and a pupal stage of 4 to 7 days.

2. Under laboratory conditions, in a constant saturated atmosphere, the percentage of larvae which transform into prepupae is not high at any temperature but reaches a maximum of 33.4 per cent. at temperatures near 21° C. The minimum effective temperature is below 7° and the maximum effective temperature is above 38°.

3. The high larval mortality is not wholly due to faulty technique,

nor to the saturated atmosphere, nor to the artificial conditions. A similar high rate of mortality is believed to occur under field conditions.

4. The percentages of prepupae and pupae which transform are high at all temperatures between the minimum and maximum effective points, and would probably be absolute under conditions of perfect technique.

5. The length of the life-cycle is decreased by increase of temperature. The average times of development plotted against temperature give, with minor corrections, an hyperbola similar to the developmental curves given by Krogh.

#### REFERENCES.

- (1) BEDFORD, H. W. (1921). The cotton thrips (*Heliothrips indicus* Bagn.) in the Sudan. *Bull.* 18, *Entom. Sect. Wellcome Trop. Res. Lab. Khartoum*.
- (2) CHAPMAN, ROYAL N. (1926). *Animal Ecology*. Burgess, Brooke Inc. Minneapolis, Min. U.S.A.
- (3) HINDS, W. E. (1903). Contribution to a monograph of the insects of the order Thysanoptera inhabiting North America. *Proc. U.S. Museum*, 26.
- (4) HORSEFALL, J. L. and FENTON, F. A. (1922). Onion Thrips in Iowa. *Bull.* 205, *Iowa Agric. Exp. Sta. Amesbury*.
- (5) VAN EECKE, R. (1922). Eerste Bijdrage tot de Kennis der Nederlandsche Thysanoptera. *Derde Verzameling Naturkundige Verhandelingen van de Wetenschappen te Haarlem*.
- (6) WILLIAMS, C. B. and KIRKPATRICK, T. W. (1924). A multiple Temperature Incubator. *Bull.* 38, *Min. Agric. Egypt, Cairo*.

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# THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

## 3. THE RELATION BETWEEN FEEDING HABITS AND PLANT LESIONS

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(With 8 Text-figures.)

THE part played by the mouthpart components of Thysanoptera, and in particular of foliage feeding Terebrantia, in producing the characteristic lesions shown by an attacked plant, and the histological nature of the lesions, are points concerning which a certain vagueness appears to exist among students of the group.

Despite the fact that the foliage lesions produced by different species of thrips do not differ greatly in type, and that there is little difference between the mouthparts of different Terebrantian species, somewhat varying explanations have been put forward.

Thus the thrips has been stated to puncture the tissues and drain the contents of the cells, causing the cell walls to collapse (Horton, J. R. 1921, re *Scirtothrips citri*. Moul.); to pierce the epidermis and rasp away the leaf tissues within (Russell, H. M. 1912, re *Heliothrips rubrocinctus* Giard.); to rasp the leaf tissues and suck up the sap as it exudes (Bedford, H. W. 1921, re *Heliothrips indicus* Bagn.); to pierce the vegetable tissue with its stylets and suck up the liberated plant juices (Cameron, A. E. and Treherne, R. C. 1918, re *Taeniothrips inconsequens* Uzel).

The experiments and observations recorded in this paper were carried out with *Thrips tabaci* under glasshouse conditions. The suitability of this species for experiments, the chief features of its life-cycle, and the degree to which it may infest the cotton plant, have already been discussed in previous papers. The plants used as host plants were cotton (*Gossypium* sp.), *Cajanus indicus* (pigeon pea, ads sudani), *Dolichos lablab* (field beans, lubia), *Hibiscus esculentus* (bamia) and *Calotropis procera* (Sodom's Apple, ushr), all grown from seed obtained in the cotton growing districts of the Sudan.

The varieties of cotton plant used were two Egyptian varieties Sakellarides and Maarad, two long staple American Upland varieties namely Webber and Cleveland Big Boll, and an unidentified short staple variety of Indian cotton.

Of these plants, the most susceptible of the cotton varieties was the Indian; then came Webber, which had, throughout the season, a mean degree of infestation approximating to 9 adults or to 16 larvae per 100 sq. cm. of leaf surface. Cleveland was less susceptible than Webber. The Egyptian varieties were less susceptible still, the mean numbers of adults and larvae per 100 sq. cm. of leaf surface, throughout the summer, being approximately two and six respectively.

Of the other plants, *Hibiscus esculentus* was almost unattacked. The leaves of this plant are very hairy and the lower surface of the leaf is covered with minute spherical exudations of numerous glands. *Dolichos lablab* was liable to slight attack when young. *Calotropis procera*, the poisonous ushr of the North African deserts, was also attacked only when young. *Cajanus indicus*, a plant that is not attacked in the Sudan by *Heliothrips indicus* and is used as a protective hedge along the windward edges of the cotton fields, was attacked freely by *Thrips tabaci*, the mean numbers of adults and larvae per 100 sq. cm. of leaf surface, throughout the period, being approximately four and ten respectively.

### **The parts of the plant attacked.**

In the case of cotton plants, *Thrips tabaci* infests the lower surface of the leaves particularly. Both adults and larvae are negatively phototropic and oviposition occurs almost invariably in the tissues of the lower surface of the leaf. The adults are slightly migratory but larvae do not leave the leaf they are born on, until they make their geotropic migration to pupate in the soil. When the lower surface of a leaf has been severely damaged and is crowded with thrip stages, some adults and larvae will migrate on to the upper surface, especially when the leaf is shaded by others, or when the sky is cloudy, or possibly in the night. Such upper surface individuals, however, are always very restless.

The preference shown for the lower surface of foliage is probably concerned with differences in epidermal thickness, between the two surfaces, rather than a question of phototropism. In cotton leaves, the difference is slight so that larvae can feed on the upper surface, but the habit has become established as an adaptation to other food plants in which this difference is more pronounced.

In the case of *Cajanus*, the usual feeding surface is the upper one,

but again thrips will occur on the other surface when crowded off the upper one. *Cajanus* is a somewhat bushy plant and the thrips frequent chiefly the lower, well-shaded leaves.

Only the leaves of the cotton plant are attacked as a rule; the bracts surrounding the boll are attacked in a few cases, and exceptionally, the bolls themselves are attacked. On American plants, the favourite situation of feeding adults and larvae is the area where the petiole joins the leaf. On Egyptian plants, the leaf edges, the area along the main veins, and the area around the marginal indentations, are all equally favoured. Both adults and larvae show a tendency to keep in the shade of the prominent veins.

Larvae occur in greater numbers on the lower, older leaves; adults are most numerous on the upper, younger leaves. Very rusty and partially withered leaves, and very young leaves, are usually free from thrips. The explanation of the latter fact is probably that such young leaves are put forth near the tip of the plant, and as infestation proceeds upwards and a normal thrip life-cycle is 3 to 4 weeks, such leaves have had time to grow considerably before the adults spread upwards to them. In severely attacked plants, where much leaf shedding has occurred, young leaves are produced low down on the stem and are then quite freely attacked.

### The mouthparts.

The mouthparts of various other species of Terebrantian thrips have been described in detail by numerous workers, notably by Jordan (1888), Garman (1890), Bohls (1891), Buffa (1898), Borden (1915), Peterson (1915), Reyne (1926), and as a careful examination of the mouthparts of *Thrips tabaci* has not disclosed the existence of any fundamental points of difference between this species and those described by the workers listed above, it will be sufficient here to limit description to certain features whose understanding is necessary to the explanation of the mode of operation of the mouthparts.

The mouthparts are bent backwards underneath the head and form a short conical proboscis or *mouthcone* (Figs. 1 and 8). This cone is directed at an angle of 45 degrees to the longitudinal axis of the body. The front of the cone is composed of a *clypeus* and *labrum* separated somewhat from one another by a membrane. Both clypeus and labrum are asymmetrical. Their shape and component sclerites are shown in Fig. 1. Each side of the mouthcone is formed by a triangular structure generally

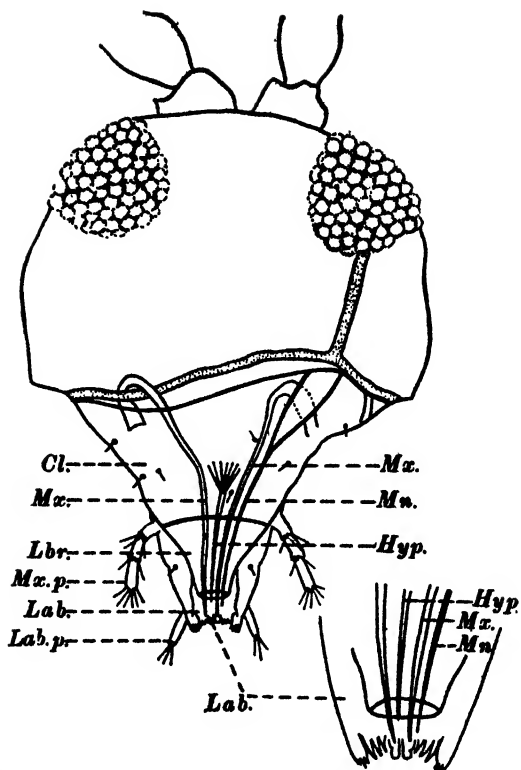


Fig. 1. Head of *Thrips tabaci*.  $\times 700$ . Cl., clypeus; Hyp., hypopharynx; Lab., labium. Lab.p., labial palps; Lbr., labrum; Mn., mandible; Mx., maxilla; Mx.p., maxillary palp.



Fig. 2. Labium of *Thrips tabaci*.  $\times 1600$ .

regarded as the galeal portion of the maxilla since it carries a three-jointed palp (Fig. 3).

The rear wall of the cone is constituted by the triangular *labium*, whose appearance is shown in Fig. 2, bearing near its tip a pair of two-jointed palps. The labium is longer than the labrum and maxillae, and projects beyond them as a flexible, somewhat fleshy flap. It may be noted that the tips of the maxillae and of the labium are provided with

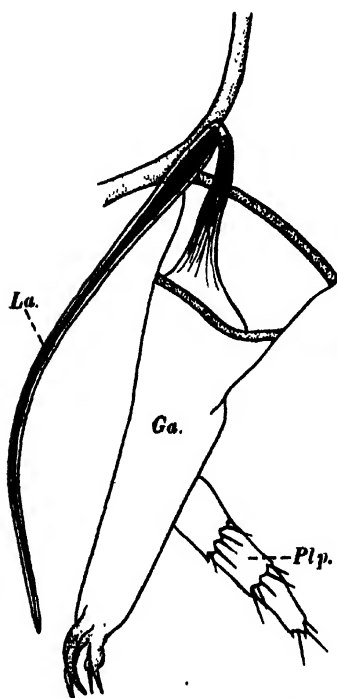


Fig. 3. Left maxilla of *Thrips tabaci*.  
× 1600. Ga., galea; La., lacinia;  
Plp., palp.

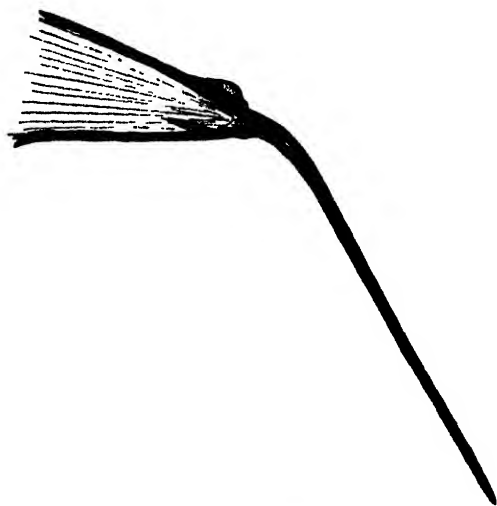


Fig. 4. Mandible of *Thrips tabaci*. × 1600.

hooks, possibly in order that this part of the mouthcone rim can grip closely the leaf surface.

The actual piercing organs comprise four *stylets* or lancets, the *paired stylets*, the *median stylet*, and the *left stylet*.

There is considerable controversy as to the homologies of these stylets. With this controversy we are not concerned. It is sufficient here to adopt the view of Reyne, the most recent investigator, and regard



the paired stylets as maxillary laciniae, the median one as the hypopharynx, and the left one as the mandible.

The shape and dimensions of these stylets may be gathered from the figures.

The method of using these stylets will be discussed later.

### **The morphological appearance of the plant lesions.**

The gradual changes in the appearance of a leaf which has been attacked by *Thrips tabaci* may for convenience be separated into four stages (Fig. 5).

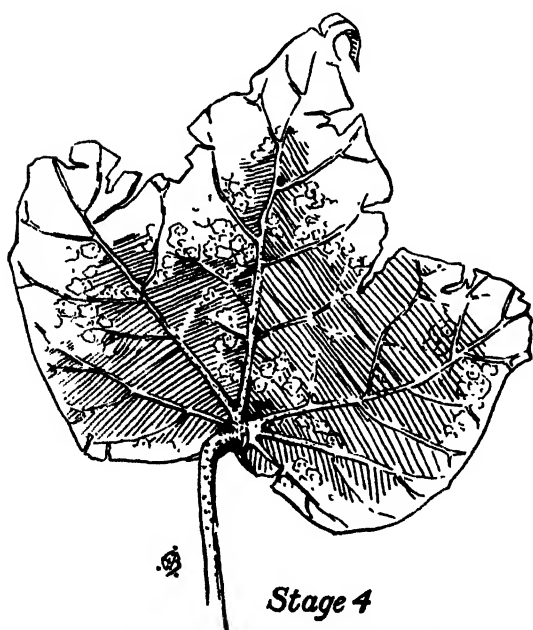
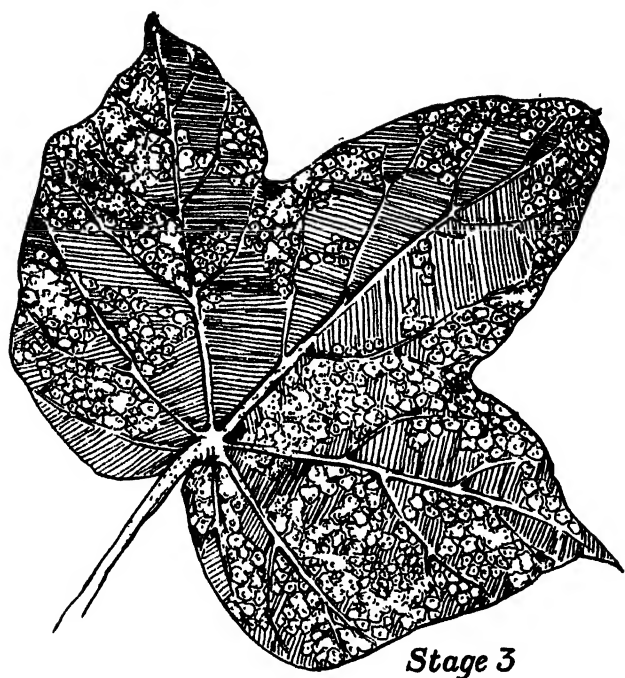
*Stage 1.* The under surface of the leaf shows a number of tiny, scattered areas which have a varnished, shining appearance, as if drops of gum arabic had coagulated on the leaf. No epidermal damage is apparent even under the microscope but each patch has a frothy, blistered appearance with interference colours. The glistening appearance is not due to a deposit on the cuticle as it cannot be removed by the action of such solvents as alcohol, ether, chloroform or xylol, nor by scraping, unless such treatment destroys the epidermis. That is to say, the shining patches do not represent a hardened exudation of sap or of insect saliva, but are due to cellular change beneath the epidermis.

Further, the appearance cannot be artificially reproduced by pricking or scraping the epidermis, nor by pricking followed by a stay of some hours in a vacuum chamber. That is to say, the assertion by Williams that, in the case of peas attacked by the Pea Thrips, the "silvering" is due to air in the outer cell layers of the plant let in by the sucking of the larva, is not wholly true, at any rate for *Thrips tabaci*. Some other cellular change in addition to the entrance of air is required.

Stage 1 damage is associated particularly with the presence of adults, and may occur often on the upper surface of leaves where neither adults nor larvae are present. In such cases, however, it is certain that the leaf surface has been visited by an adult thrips.

*Stage 2.* Within 24–48 hours the patches take on a greenish brown colour and become much more conspicuous.

*Stage 3.* The brown spots become larger, more scattered over the leaf surface, particularly occurring along the course of the main veins. With Egyptian plants, these patches are pale brown and more frequently found on the upper leaf surface than in the case of American plants. If pieces of the damaged leaf at either Stages 2 and 3 be taken, soaked in an oxydising agent such as nitric acid plus potassium chlorate, or sodium hypochlorite, and, when transparent, transferred to cold water,



**Fig. 5.** Leaves of Sakellarides cotton showing Stage 3 and Stage 4 damage by thrips.

it is possible to remove the piece of epidermis over the damaged area. To such pieces of epidermis the superficial layer of mesophyll cells will adhere at the points of damage but not elsewhere. Such pieces can be conveniently stained with Bismarck brown.

Careful examination of such pieces under a high power binocular microscope reveals nothing in the nature of punctures, but shows a number of gashes in the damaged areas, each gash within the boundaries of a single epidermal cell (Fig. 6).

Around each gash, the epidermal cells are intact but cover a little patch of somewhat disorganised mesophyll cells.

There is little doubt that the brown appearance of the patches in a Stage 2 or Stage 3 leaf is due to necrosis of the more superficial mesophyll layers, and that these brown patches underlie the epidermis. It is the lens-like effect of the transparent epidermis overlying a patch of

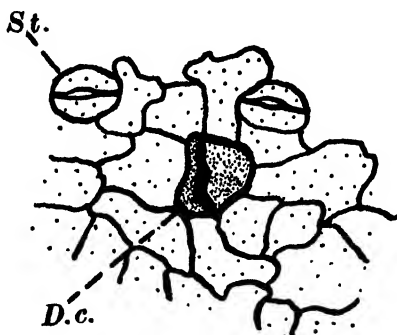


Fig. 6. Portion of epidermis from lower surface of a cotton leaf, to show the appearance of a cell gashed by a thrips.  $\times 700$ . *St.*, a stoma; *D.c.*, damaged cell.

shattered mesophyll cells that produces the glistening, colour-fringed effect of Stage 1 damage.

Necrosis is probably a purely mechanical sequel to insect damage, and not aided by any toxic salivary secretion. Similar brown patches can be produced, but not glistening patches, by pricking the epidermis with a sterile needle. They can be produced by pricking with a sterile needle which has been dipped in an infusion of damaged leaves made by grinding up such leaves with sterile sand and distilled water. In neither case does damage spread beyond the little patch of dead mesophyll cells which results in the neighbourhood of the puncture.

Similar spots produced by an isolated adult are a little larger than the artificially produced ones but again do not spread greatly in area, whereas, if the salivary secretion of the insect were toxic, one would

expect extensive spreading of the damaged spot even after the insect had been removed. Actually, only when several insects are allowed to roam about the leaf for some time, can one obtain spots sufficiently close together to merge into brownish areas.

In this case, the merging of the spots is aided by a general necrosis of the leaf caused by interference with transpiratory and photosynthetic functions.

*Stage 4.* The patches tend to run into one another so that the under surface of the leaf becomes uniformly purplish or rusty brown. On Egyptian leaves, the colour is a light brown and the veins are bordered by reddish brown patches. The upper surface of the leaf becomes dry, harsh and wrinkled. The Stage 4 leaf in fact is with difficulty distinguishable, as regards colour changes, from a normal dying or dead leaf. Necrosis however has involved the epidermal cells and these are broken and shapeless. Thrips occur rarely on Stage 4 leaves.

The rapidity with which Stage 4 is reached after initial infection, depends upon the degree of infestation to which the leaf has been subjected. The usual length of time in the case of American leaves was ten days. A large number of experiments were made in the confinement of known numbers of adults or larvae upon leaves for varying intervals of time, upon marked and isolated leaves. The results obtained indicated:

(a) That one thrips alone can bring about Stage 1 damage.

(b) That if no further feeding upon the leaf is allowed, the leaf being segregated so far as possible by removal of all leaves in its vicinity, damage does not proceed beyond Stage 2.

(c) That only when the attack is cumulative and severe, does the leaf assume the appearance of Stages 3 and 4.

Presumably when only a few cells are tapped, necrosis of the leaf does not proceed beyond the damaged points and premature falling of the leaf does not occur. If a large number of cells are tapped, however, the whole leaf becomes uniformly withered and leaf shedding occurs long before the leaf would normally fall.

It is probable however that different varieties of the cotton plant will vary as regards susceptibility to thrip injury. Reyne (1921) states that there is great difference in behaviour of food plants with regard to the injury caused by the Cacao Thrips (*Heliothrips rubrocinctus*); in Cacao, *Mangifera*, *Anacardium*, necrosis of the feeding marks occurs and the leaves are cast; in this case severe damage is done as the trees soon become exhausted by accelerated change of leaf; in others (*Ter-*

*minalia*, Biza), the leaf tissue does not die and premature leaf shedding does not occur.

Damage is produced by both adult and larval *Thrips tabaci*. Leaves on which only larvae were confined deteriorate much more slowly than those upon which adults only are confined. The latter leaves pass rapidly into Stage 2 and reach Stage 3 whilst the former leaves are only slowly approaching Stage 2. The appearance of the damage produced by adults is indistinguishable from that produced by larvae.

### **The histology of the lesions.**

Pieces of leaf were fixed in weak Fleming's solution; fixation was carried out in partial vacuo, as described by other workers. The results with cotton leaves are not so satisfactory as those obtained by fixation with Carnoy's fluid.

Sections of thickness  $10\mu$  to  $12\mu$ , and  $18\mu$  to  $20\mu$ , were cut; the thicker sections were more reliable for purposes of examination. Of a large range of stains and combinations of stains used, the most satisfactory results were obtained with iron haematoxylin, and with aniline blue and cotton red in combination. Bismarck brown was used for staining portions of epidermis or cuticle. Sections were cut from three classes of material, namely:

- (i) fresh undamaged leaves and normal withered leaves,
- (ii) leaves in the four stages of thrip damage,
- (iii) leaves on which known numbers of thrips had been confined for known periods of time.

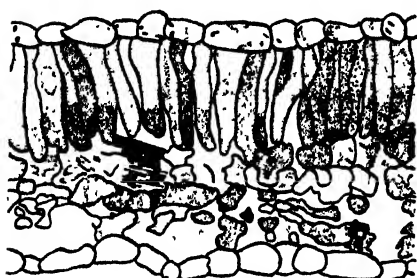
The conclusions derived from examination of several thousands of such sections may be summarised as follows (Fig. 7):

*Stage 1.* The epidermis is intact on both sides, except for short breaks in the lower epidermis. There is no evidence of punctures such as Reyne (1926) claims are visible in the epidermis of leaves of Liberian coffee attacked by *Heliothrips haemorrhoidalis*. Below the epidermal breaks, and below intact portions of the epidermis, where the section has possibly passed to one side of a break, there is slight disorganisation of the outermost mesophyll layer, some of whose cells appear to be shattered and emptied, and an unusually large number of air spaces. The palisade layer is always intact.

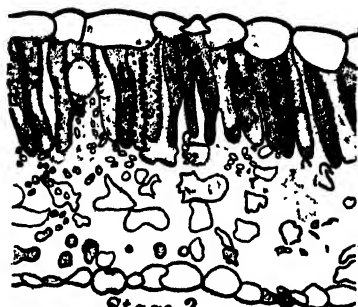
*Stage 2.* The appearance of this stage is similar to that of Stage 1, but cells of the lower epidermis are in places somewhat misshapen. Distortion of patches of the outermost mesophyll layer is still more

obvious and large spaces occur where the cells are destroyed or emptied and more or less shrivelled.

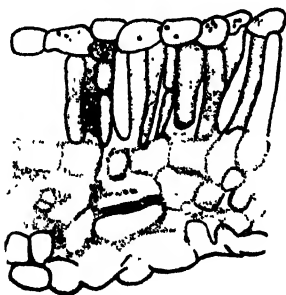
*Stage 3.* The whole section has a shrivelled appearance. The mesophyll is so disorganised as to appear almost unrecognisable in places. Disorganisation has spread also to the palisade tissue but the upper epidermis is still fairly intact.



*Stage 1*



*Stage 2*



*Stage 3*



*Stage 4*

Fig. 7. Histological appearance of the four stages of thrip damage to cotton leaves.  
× 500.

*Stage 4.* All the tissues are disorganised. The mesophyll is completely broken down. Only the outlines of the palisade layer remain. Both epidermal layers are broken down.

That is to say, the histological appearance of damaged leaves suggests that the damage caused is of a purely mechanical nature. The appearance of Stage 4 leaves is in no way different from that of undamaged leaves which have gradually withered.

The histological appearance of leaves upon which adults or larvae had been separately confined differed in no way from one another.

**Conclusions concerning relation between the mode of feeding and the leaf lesions.**

The following conclusions are based upon close observation of feeding adults and larvae, microscope examination of the mouthparts of adult and larval stages, and microscopical examination of damaged leaves. The method used by some workers when studying the feeding habits of Hemipterous insects, of fixing the feeding insect to the leaf by a drop of wax and then preparing insect and piece of leaf for section cutting, was tried with *Thrips tabaci* but was found useless. The mouthparts do not penetrate sufficiently deeply into the leaf tissues to ensure that the insect will remain in position after the wax has become dissolved by the fixative. Despite the greatest care in the subsequent technique, it was not possible to keep a single specimen in position, although perfectly easy to anchor an ovipositing adult by the ovipositor.

Cotton leaves vary of course in thickness with age, size, variety, and water supply. The proportions of the various histological layers to one another, however, may be indicated by the following measurements which represent means of a large number of leaf measurements. When the mean thickness of the leaf is  $143\mu$ , the upper epidermis is  $17\mu$ , the palisade tissue  $54\mu$ , the mesophyll layers  $61\mu$ , the lower epidermis  $11\mu$ , respectively. The lower mesophyll layers are somewhat loosely packed, a feature which varies considerably in different varieties of the cotton plant, and appears also to vary according to the intensity of light to which the leaf happens to have been subjected.

On the lower surface occur numerous hairs. There is no evidence that the presence of such hairs is repellent to thrips. The spacing between the hairs is sufficiently wide to permit such minute insects as Thysanoptera to move about freely. In fact the hairy Indian and American plants were more infested than were the smoother leaved Egyptian plants. It is possible however that the closer spacing of the fine hairs on the lower surface of *Cajanus* leaves is a factor in producing the relative freedom of this surface from thrip attack.

The method of feeding appears to be as follows:

The mouth cone is applied firmly to the leaf surface, the animal sinking somewhat between the anterior legs so that the abdomen is tilted upwards. The hooked tips of maxillae and labium grip the leaf surface firmly, but since the rear wall of the cone is longer than the fore wall, and flexible, a slight rocking movement of the head is still possible. The stylets, at any rate the maxillae and the mandible, can be protruded apparently through a circular rim at the tip of the labrum.

The distance to which they can be protruded is not easy to estimate, since in microscopical preparations there is the possibility that pressure on the cone has brought about abnormal length of protrusion. In the case of *Thrips tabaci*, the distance to which the stylets can be protruded beyond the labral rim appears to be approximately  $11\ \mu$  for the mandible, and  $27\ \mu$  for the maxillae. Whether the hypopharynx can be protruded also is uncertain. Borden claims so, for *Heliothrips haemorrhoidalis*, and possibly such is also the case with *Thrips tabaci*.

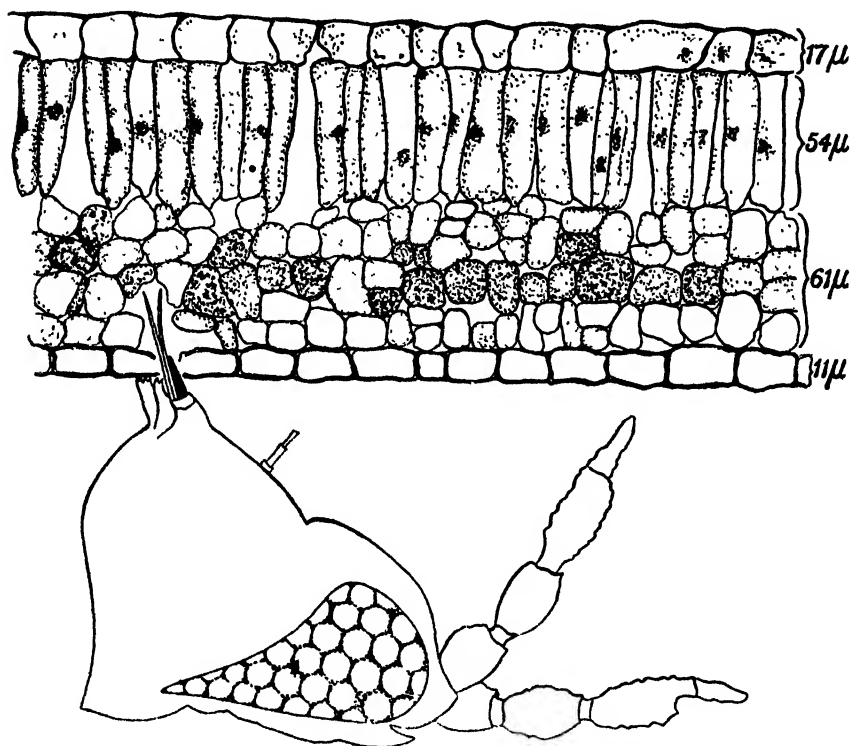


Fig. 8. Diagram of the head of a feeding thrips to indicate its size relative to the leaf tissues, and the depth to which maxillae and mandible are inserted.

What exactly occurs, during feeding, within the semi-enclosed chamber formed by apposition of the mouth cone to the leaf surface, is difficult to follow, but seems to be somewhat as follows.

The feeding thrips commences to swing the head slightly upwards and downwards, the flexibility of the labium permitting this to occur without disturbance of the position of maxillae and labium. The rocking



movement is made possible by the triangular shape of the head (Fig. 8). At each swing upwards, when the thrips is on the lower surface of the leaf, as in the figure, the mandible is protruded, at each swing downwards it is withdrawn; the insect thus uses the mandible as a man uses a pickaxe, and its chief function appears to be that of breaking through cuticle and outer epidermal wall. That is to say, it gashes or tears rather than pierces. The arc subtended by the angle of swing is limited by the degree of flexibility of the labium. It is in any case small, since the gash is within the length of an epidermal cell, that is to say within a length of  $50\mu$ , although in some cases it may cut across the dividing wall between two cells. The protruded mandible will in most cases not be long enough to reach the lower wall of the epidermis. When the upper surface is broken through, the longer and weaker maxillae come into play, the head swings lessen somewhat, and the boundary between epidermis and outermost mesophyll layer is broken down. Probably the lateral boundary walls of a number of mesophyll cells are broken down, since these cells are smaller than those of the epidermis, and a number of them will underlie one epidermal cell.

There is no evidence that the mouth cone is pushed down into the gash thus created, or that it can be pushed down into a stoma and the leaf tissues attacked that way. The rocking movements, essential apparently to the use of the stylets, would be impossible if the cone became wedged into a gash or into a stoma. The feeding habits of the insect will be determined by the length to which it can protrude its stylets, and the epidermal thickness of host plants. The variation in susceptibility of individuals in a block of plants of the one variety, ranging as it does from very slight injury to severe injury, will arise almost entirely through variations in epidermal thickness between leaves of different plants, and in variations in age and size of the thrip stages on the plants.

After the gash is made, the head movements cease and the thrip remains apparently motionless, absorbed in the effort of sucking inwards the chloroplasts of one or more mesophyll cells. The passage of chloroplasts into the pharynx is aided by the partial vacuum created in the closely applied mouth cone. Usually, after gashing one epidermal-cell and absorbing the contents of the underlying mesophyll cells, the insect travels on and gashes another cell further away, following usually the course of a venule.

At first, the wound shows on the leaf as a glistening spot, the effect being due to the lens-like effect of the transparent epidermis over the empty patch of mesophyll cells underlying the gash. Then, as the patch

of mesophyll undergoes necrosis, the spot turns brown. Slight spreading of the necrosed spots, and the confluence of a large number of these spots, produces the Stage 3 damage. Gradual death of the injured leaf brings about the appearance of Stage 4.

To the list of acknowledgments already given in the first part of this paper, we would add the following names: Mr C. C. Brooks for very great help in the preparation of thrip mouthparts; Mr Howarth, Mr Walton and Mr Sutton of the Botanical Department staff of this University, and Dr K. Smith of the Agricultural staff, for advice concerning botanical technique.

#### SUMMARY.

1. Observations and experiments concerning *Thrips tabaci* on cotton and other plants suggest that plant injury from thrip attack is in this case due entirely to premature and excessive defoliation, and is dependent in extent and severity upon the value of the infestation factor, or number of thrip stages per unit area of foliage surface. Differences between plant species, plant varieties and plant individuals as regards susceptibility to injury arise chiefly through variations in thickness of the epidermal cell layer.

2. Leaf injury consists essentially of necrosis of a patch of mesophyll cells lying immediately below a gash in an epidermal cell. Such necrosed patches are scattered and isolated when the infestation factor is low, but are confluent and form extensive rusty areas when the infestation is high. Such areas consist of dead cells of the superficial mesophyll layers; the deeper layers, palisade tissue and epidermal layers remain intact, except for the initial gashes made in epidermal cells. In later stages of leaf injury, such necrosis involves the whole leaf, all the layers become disorganised, and premature shedding occurs. There is no evidence that the insect salivary secretion is toxic.

3. The insect does not puncture or rasp the epidermis but gashes an epidermal cell by pickaxe-like movements of the single mandible, induced by a slight rocking movement of the head. In most cases, the mandible can only gash the outer epidermal wall, the inner wall and the lateral walls of the mesophyll cells being broken down by similar movements of the longer protruded maxillary laciniae. There is no evidence of attack through the stomata. Suction of the chloroplasts into the pharynx is aided probably by the partial vacuum established within the mouth cone when applied closely to the leaf surface.

4. The preference shown by thrip stages for the lower surface of leaves is believed to be due rather to differences in thickness of epidermis between the lower surface and the upper surface, than to negative phototropism. In plants where such differences are slight, such as cotton plants, thrip stages readily invade the upper leaf surface. On *Cajanus indicus*, the upper surface is the more favoured, the lower surface being unsuitable owing to the close spacing of numerous fine hairs. The more widely spaced hairs on cotton leaves do not act as a deterrent, the hairy American and Indian varieties being more heavily attacked than the smoother leaved Egyptian varieties of cotton.

## REFERENCES.

- (1) BEDFORD, H. W. (1921). The Cotton Thrips (*Heliothrips indicus* Bagn.) in the Sudan. *Bull.* 18, *Entom. Sect. Wellcome Trop. Res. Lab. Khartoum*.
- (2) BOILLS, J. (1891). *Die Mundwerkzeuge der Physapoda*. Inaug. Dissert. Göttingen.
- (3) BORDEN, A. D. (1915). Mouthparts of Thysanoptera. *Journ. Econ. Ent.* viii.
- (4) BUFFA, P. (1898). Contributa allo studion anatomia della *Heliothrips haemorrhoidalis*. *Revista di Patologia Vegetale*, viii.
- (5) CAMERON, A. E. and TREHERNE, R. C. (1918). The Pear Thrips (*Taeniothrips inconsequens* Uzel). *Bull.* 15, *Ent. Br. Dept. Agric. Dominion of Canada*.
- (6) GARMAN, H. (1890). The mouthparts of Thysanoptera. *Bull. Essex Institute*, xxii, and *Canadian Entomologist*, xxii.
- (7) HORTON, J. R. (1918). The Citrus Thrips. *Bull.* 616, *U.S. Dept. Agric.*
- (8) JORDAN, K. (1888). Anatomie und biologie der Physapoda. *Zeitschr. f. Wiss. Zool.* xlvii.
- (9) PETERSON, A. (1915). Morphological studies on the head and mouthparts of Thysanoptera. *American Naturalist*, xxx.
- (10) RUSSELL, H. M. (1912). The Red-Banded Thrips. *Bull.* 99, *U.S. Dept. Agric.* Part II.
- (11) REYNE, A. (1921). De Cacaothrips (*Heliothrips rubrocinctus*. Giard.). *Bull.* 44, *Dept. v. den Landbouw, Suriname*.
- (12) — (1926). *Onderzoekingen over de monddeelen der Thysanoptera*. Dissertation for Doctorate in the Univ. of Utrecht.
- (13) WILLIAMS, C. B. (1915). The Pea Thrips (*Kakothrips rosustus* Uzel). *Ann. App. Biol.* i.

(Received 24th June, 1927.)

## PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY meeting held at 2.30 p.m. on May 13th, 1927, in the Imperial College of Science. The President, Mr J. C. F. FRYER, M.A., in the Chair.

### DISCUSSION ON PLANT ALKALOIDS.

- I. "The Principal Plants yielding Alkaloids" by Lieut.-Colonel A. T. GAGE, C.I.E., lately Director, Botanical Survey of India, and Superintendent, Royal Botanic Gardens, Calcutta.
- II. "The Biochemistry of the Alkaloids" by Dr T. A. HENRY, Director, The Wellcome Chemical Research Laboratories.
- III. "The Medical Aspects of the Alkaloids" by Dr J. TREVAN, The Wellcome Physiological Research Laboratories.
- IV. General Discussion—Mr FRYER, Dr PETTYBRIDGE, Mr HOWES, Dr HENRY, Dr TREVAN.

### I. THE PRINCIPAL PLANTS YIELDING ALKALOIDS.

By Lieut.-Colonel A. T. GAGE, C.I.E., M.A., M.B., B.Sc.

*(Late Director, Botanical Survey of India, and Superintendent,  
Royal Botanic Gardens, Calcutta.)*

MAJOR CHIPP in his letter in which he conveyed his honourable invitation for me to take part in this alkaloidal symposium had the following remark: "We should not trespass on your time for more than twenty minutes." In this there was an obvious transposition of the pronouns, for he should have written "You should not trespass on our time for more than twenty minutes." Anyhow I hope I shall not much exceed that limit.

Fortunately for you and me I am not called upon to say anything about alkaloids themselves. I am merely the showman pointing to the pictures with more or less appropriate patter, to whet your appetite for the real performance, when Drs Henry and Trevan put their trained troupe of alkaloids through their paces.

I may just confide in you my personal impression of the rarity of alkaloids in their unadorned beauty. We consume incredible quantities of tea, coffee and tobacco, but how many of us have ever seen Caffeine or Nicotine. For half of every year for many years I lived amidst miles of Tea and Cinchona plants, but only on one occasion have I been introduced to Caffeine and Quinine, when the Government Quinologist showed me two phials, one of which, he said, contained Caffeine from tea-prunings and the other Quinine. I inspected them with the reverential care they seemed to call for and handed them back to him. Since then I have seen no other.

As within the time at our disposal only a limited number of the regiment of alkaloid-yielding plants can be inspected, the selection of those to file past leaves

room for difference of opinion according as to whether the arranger of the inspection is an economic biologist, a pure chemist, a pharmacologist, a physiologist, a physician, a grocer, a tobacconist or an artist in poisoning.

Although it is possible that not all of these are represented here, there is probably enough of different interests to make the selection somewhat of a problem. Being unfortunately precluded from adopting one of the bright modern methods of simultaneously raising money and gaining information by inviting you to participate on the usual terms in an "Alkaloid Ballot," I have fallen back upon a method of the B.B.C. who occasionally broadcast a programme arranged by an outsider according to his individual taste. So as one who is, or rather was, a mere systematic botanist, I have made out my own list with an equitable disregard of everyone else's interest. The question of the relative importance of alkaloids is as far as I am concerned evaded by passing the plants along in the order of the natural families to which they belong. At the risk of trespassing on Dr Henry's province, it may be mentioned here that when, as the slides are passing along, only one alkaloid is mentioned in connection with a plant it is not to be inferred that the plant contains no others. Further, when roots, seeds, bark or other parts are mentioned as the source of alkaloids, it means merely that the alkaloids are usually or most conveniently extracted from such parts. Finally such expressions as root or seed or bark and suchlike are extremely crude indications of how the protoplasm of a plant disposes of the alkaloids it produces in the course of its metabolism.

Lantern slides were then shown of the following plants, the lecturer commenting upon each.

1. *ACONITUM NAPELLUS* L. (Ranunculaceae), the familiar Monkshood of our gardens, is taken as the representative of a genus widely distributed over the Northern Temperate Zone. Most of the species store alkaloids, chiefly in the root, and several of these alkaloids are very poisonous. The typical alkaloid is Aconitine.

2. *BERBERIS ARISTATA* DC. (Berberidaceae) is a spiny evergreen shrub, a native of the Himalayas and the hills of Southern India and Ceylon and cultivated in this country as an ornamental shrub. The thin brittle root-bark more particularly yields *Berberin*, an alkaloid that occurs in a considerable number of other species, some belonging to the same genus, and others to different families.

3. *PAPAVR SOMNIFERUM* L. (Papaveraceae), the opium poppy, is probably a native of S.E. Europe and Asia Minor, but cultivated in Asia Minor, Persia, India still to a small extent, and China. The milky juice, which is collected by incising the capsules, is a fount of different alkaloids, over a dozen occurring in the stream. The chief one is of course Morphine.

4. *CAMELLIA THEA* (Ternstroemiaceae). The tea plant is native to Assam and extensively cultivated in that province, the Eastern Himalayas, Southern India, Ceylon, Java, China and elsewhere in the Tropics and Sub-tropics. It contains the most widely consumed of all alkaloids in the form of caffeine, which is usually extracted from the leaves.

5. *THEOBROMA CACAO* L. (Sterculiaceae). The cocoa plant is a small tree, a native of the northern parts of South America and up to as far north as Mexico. Extensively

cultivated in the tropics. The alkaloid Theobromine is the substance in the seeds which, along with the concrete oil, gives the flavour to the beverages that Linnaeus considered such divine liquors that he called the genus *Theobroma*, the Food of the Gods.

6. *ERYTHROXYLON COCA* Lamk. (Linaceae), the source of Cocaine, is a shrub extensively cultivated in the northern Andes and also in Brazil and other South American countries. The alkaloid that is extracted from the leaves reminds me that I might have included detectives in the list of the interested ones, as it is probably better known by repute than any other alkaloid to the ordinary man from its association with Sherlock Holmes in literature and Scotland Yard in newspaper reports of the activities of "snow" smugglers.

7. *PILOCARPUS PINNATIFOLIUS* Lemaire (Rutaceae), from the leaves of which the alkaloid Pilocarpine is obtained, is a shrub of Brazil. The leaves are compound, a foot or more long, with 2-5 pairs of 3-4 in. long leaflets and a terminal one. The inflorescence of long spiciform erect racemes, bearing numerous shortly pedicelled small reddish purple flowers.

8. *PHYSOSTIGMA VENENOSUM* Balf. Calabar Bean. A large leguminous climber, a native of Old Calabar, but introduced into Brazil and India. The seeds contain the well-known alkaloid Physostigmine or Eserine, very poisonous.

9. *CONIUM MACULATUM* L. (Umbelliferae). Hemlock. This herb has a wide distribution throughout Europe to temperate Asia and North Africa and has been introduced into both North and South America.

The species harbours several alkaloids. In poisonous doses the mind remains unaffected to the last, the classical instance of this being the death of Socrates as related so movingly by Plato in the *Phaedo*.

10. *CINCHONA LEDGERIANA*, *C. OFFICINALIS* and *C. SUCCIRUBRA* (Rubiaceae). Trees native to the eastern side of the Andes and introduced some 60 years ago into Java and India. They contain several alkaloids, the most important from the commercial and medical point of view being Quinine. The most important species is *C. Ledgeriana*, by far the most extensively cultivated.

11. *COFFEA ARABICA* and other species (Rubiaceae). A shrub or small tree native to tropical Africa, but widely cultivated in the tropics. The characteristic alkaloid obtained from the seeds is Caffeine, which occurs also in Tea, Paraguay Tea (*Ilex paraguayensis*), Kola and other species.

12. *PSYCHOTRIA IPECACUANHA* (Rubiaceae). A small shrub, a native of the tropical forests of Brazil, but cultivated in other parts of the tropics. It is cultivated to a small extent in India and still more in the Malay Peninsula.

Emetine, the alkaloid so useful in one form of dysentery, is contained chiefly in the root bark.

13. *STRYCHNOS NUX VOMICA* L. (Loganiaceae). A moderate sized tree, native to India, Ceylon, Burma, Cochin-China and parts of the Malay Archipelago. The seeds yield several alkaloids, the chief being Strychnine. Taste is very bitter. CURARINE got from other species of *Strychnos*.

14. *NICOTIANA TABACUM* L. (Solanaceae). A coarse annual. Probably a native of Central or South America.

The leaf alkaloid is a volatile liquid, Nicotine.

15. *DATURA STRAMONIUM* L. (Solanaceae). A coarse weedy annual. Spread throughout the world. Daturine from the leaves and seeds. Akin to Atropine.

16. *ATROPA BELLADONNA* L. (Solanaceae). A large bushy herb. Central and Southern Europe, South-west Asia and North Africa.

ATROPINE from the roots and leaves. Crystalline.

17. *HYOSCYAMUS NIGER* L. (Solanaceae). An annual or biennial herb. Temperate Europe and Asia. Introduced into America.

Hyoscyamine from the seeds and leaves.

## II. THE BIO-CHEMISTRY OF ALKALOIDS.

By T. A. HENRY, D.Sc. (London).

(*Director Wellcome Chemical Research Laboratories.*)

THE first clearly defined alkaloid—morphine—was isolated in 1817, so that over a century has elapsed since the interest of chemists in these products was first evoked. During that period hundreds of new alkaloids have been discovered and described, and great progress has been made in the determination of their intimate structure. The precision which has been reached in some branches of work of this kind may be gauged from the fact that last year conclusive proof of the constitution of the rare opium alkaloid, codamine, was furnished by Prof. E. Spath of Vienna by the use of only 0.2 gm. of the original specimen of this alkaloid isolated from opium by O. Hesse in 1870.

The constitutions of quinine and the more important related alkaloids derived from cinchona have been known for some years, and syntheses of this group of alkaloids cannot now be long delayed. The structure of all the commoner alkaloids of opium is known, with the exception of the most important of all, viz. morphine and its close relatives, codeine and thebaine, and here only details remain to be settled. Similar progress is being made with other alkaloids of special therapeutical interest such as emetine and strychnine, and the activity in alkaloidal research at Manchester, Oxford, Marburg and Vienna is such that one wonders how long the known supply of "alkaloids of unknown constitution" will hold out. The organic chemist can reasonably claim that he has done his part in adding to the knowledge of alkaloids, but it is of interest to note that in spite of all this activity no natural alkaloid has yet been replaced commercially by its synthetic equivalent, though this could be done in a number of cases if necessity for it arose. The fact that man is still dependent on natural sources for supplies of these indispensable medicinal products makes it all the more surprising that next to nothing is known of the part they play in the physiology of plants. This lack of knowledge is no doubt due to the facts that the most important alkaloidal plants are grown in the Tropics, where facilities for bio-chemical research are still severely restricted, and that, except in the cases of cinchona and

opium, production is scattered and on a small scale, so that there is no organisation for the systematic research necessary to secure such information. Alkaloidal plants, such as those of the Solanaceae and the Leguminosae are however available in temperate regions, and the numerous problems presented by the distribution, origin and function of alkaloids in plants will no doubt eventually attract the attention of the bio-chemist. At present the bio-chemistry of alkaloids consists of little more than casual, unrelated observations with much, more or less, plausible speculation.

Alkaloids may be defined as naturally occurring, relatively complex, basic substances usually exhibiting marked physiological action, but the term is not always used in this strict sense outside chemical circles. It is not uncommon to find the name applied to such substances as digitoxin, strophanthin and santonin, that is, to naturally occurring, physiologically active substances, which contain no nitrogen and are not basic. That is the essential difference and, broadly speaking, alkaloids are classified for convenience of description according to the way in which the constituent nitrogen is built into the molecule. The simplest alkaloids are derivatives of open chain or aliphatic amines, whilst the more complex have as nuclei, closed heterocyclic chains in which the nitrogen is the heterogeneous element. The more complex alkaloids are further subdivided, according to the nature of these heterocyclic rings into derivatives of pyrrolidine, piperidine, glyoxaline, etc., and still further according to the nature of the proximate structure of which the primary heterocyclic ring forms part, into tropane, granatane, quinuclidine, quinoline, isoquinoline, indole, purine etc., derivatives. Such schemes of classification become increasingly difficult as knowledge of the structure of alkaloids grows and though they are always misleading something of the kind is necessary for purposes of general discussion.

Taking a group of plant species so closely related as to be included in one natural order, it is reasonable to assume that the plants concerned will possess a common metabolism and have most of their characteristic constituents in common. That seems to be true as regards alkaloids in only one natural order, viz. Amaryllidaceae in which the characteristic alkaloid is an isoquinoline derivative, lycorine or narcissine which has been found in at least ten genera of the order. It is also true of such a characteristically alkaloidal order as the Papaveraceae where in spite of the many different alkaloids that may be present in a single plant—there are at least 25 in the opium poppy—all are isoquinoline derivatives, so far as is known, and all can be regarded as arising by a common process.

A more usual state of things is presented by such orders as the Solanaceae and the Leguminosae. In the former three well-defined and distinct groups of alkaloids occur, typified by hyoscyamine (tropane nucleus), nicotine (piperidylpyrrolidine nucleus) and solanine (a complex alkaloidal glucoside of unknown constitution) respectively. Hyoscyamine occurs in several genera, *Datura*, *Atropa*, *Scopolia*, *Duboisia* and *Hyoscyamus*, nicotine in one only, *Nicotiana*, whilst the solanines are found only in *Solanum* species.

Much the same state of things is found in the Leguminosae where cytisine (quinoline condensed with pyrazine or pyrazole as a nucleus) is the most widely distributed alkaloid having been found in *Anagyris*, *Baptisia*, *Cytisus Laburnum*, *Euchresta*, *Genista*, *Sophora* and *Ulex*. The alkaloid sparteine, which is a quinuclidine derivative, a characteristic which it shares with the principal cinchona alkaloids, is found in the genus *Lupinus* and in one species of *Cytisus* (*C. scoparius*). Trigonelline,



a simple pyridine derivative, is found in *Trigonella* and in *Pisum*. Hypaphorine and eserine, respectively simple and complex derivatives of indole occur in *Erythrina hypaphorus* and *Physostigma Venenosum*. Finally vicine, probably a purine glucoside, occurs in *Vicia* spp. and there are less well-defined alkaloids in other genera of the order. In view of this variation in the type of alkaloid, which may occur within a single natural order or even within a genus, as in *Cytisus*, it seems clear that by whatever process alkaloids may be formed in plants, it must be a process which from similar materials must be capable of giving rise to a variety of products.

None of the alkaloids so far mentioned are to be found in plants of more than one natural order, but berberine, one of the best known of the isoquinoline alkaloids, has been recorded in at least 14 genera belonging to five natural orders, Ranunculaceae, Berberidaceae, Menispermaceae, Papaveraceae and Rutaceae.

It is common ground with authors, who have put forward suggestions as to the mode of origin of alkaloids in plants that the primary materials must be amino-acids, produced by the breaking down of proteins, but it is at least equally probable that they are formed as by-products in the building up of proteins. The former process can be demonstrated, so far as the production of simple bases—too simple to be regarded as alkaloids—is concerned from what is known regarding the hydrolytic products of gliadin, one of the proteins found in rye and the amino-acids and amines found in ergot of rye and presumably produced by the action of this fungus on the proteins of rye grain. As shown in the following table, the six amino-acids from leucine to lysine are all found among the hydrolytic products of the gliadin of rye, whilst in the fungus the same amino-acids are represented by the corresponding amines, isoamylamine to cadaverine, produced in each instance by decarboxylation of the amino-acid, of which in most cases some also survives in the fungus.

*Simple Bases of Ergot of Rye.*

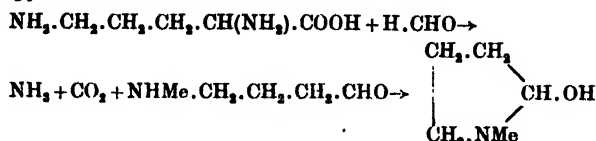
1. Leucine	$\text{CHMe}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$	in rye and ergot
isoAmlyamine	$\text{CHMe}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$	in ergot
2. Aspartic acid	$\text{COOH} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$	in rye and ergot
3. Histidine	$  \begin{array}{c}  \text{CH} - \text{NH} \\  \diagup \quad \diagdown \\  \text{C} \cdot \text{CH}_2 \text{CH}(\text{NH}_2) \cdot \text{COOH} \\  \text{N} - \text{CH} \\  \diagdown \quad \diagup \\  \text{CH} - \text{NH} \\  \diagup \quad \diagdown \\  \text{C} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2 \\  \text{N} - \text{CH}  \end{array}  $	in rye and ergot
Ergamine		in ergot
4. Tyrosine	$\text{HO} \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$	in rye and ergot
Tyramine	$\text{HO} \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$	in ergot
5. Arginine	$\text{NH}_2 \cdot \text{C}(\text{NH}) \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$	in rye
Agmatine	$\text{NH}_2 \cdot \text{C}(\text{NH}) \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$	in ergot
Putrescine	$\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$	in ergot
6. Lysine	$\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$	in rye
Cadaverine	$\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$	in ergot

This sort of process gives us no clue to the origin of the real alkaloids, ergotoxine and ergotamine, found in the fungus and to which there is nothing analogous among the constituents of rye grain or their hydrolytic products.

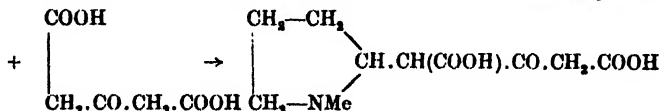
Of all the suggestions that have from time to time been put forward to account for the formation of alkaloids the most useful is that published by Robinson in 1917. These views have the great advantage that they postulate the pre-existence in the plant of nothing not known to occur there, or at any rate not likely to be present, they assume only the possibility of reactions, which there is every reason to believe do occur in plants, and Robinson has himself shown by the synthesis of tropinone and pseudo-pelletierine in the laboratory by these processes, that the latter can take place at atmospheric temperature.

On the basis of these views the following explanation of the formation of hygrine, the simplest alkaloid found in coca leaves is given.

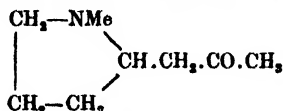
*Stage I.* Oxidation and methylation of ornithine by formaldehyde to a carbinolamine of the pyrrolidine series.



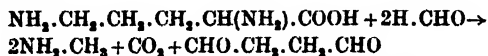
*Stage II.* Condensation of the carbinolamine with acetonedicarboxylic acid.



*Stage III.* Decarboxylation of the condensation product to (in this case) hygrine.



By a similar condensation of two molecules of the carbinolamine with one molecule of acetonedicarboxylic acid, a base isomeric with cuskhygrine would be produced, whilst, if Stage I is carried a step further, ornithine might yield succindialdehyde and methylamine, thus:



and from these two substances and acetonedicarboxylic acid, Robinson has shown that tropinone can be produced in the laboratory, and tropinone is a probable intermediate in the production of tropine and eventually of hyoscyamine in the plant.

Still keeping to the same materials, ornithine and acetonedicarboxylic acid, it is possible to represent by reasonable variations in the same processes the formation of nicotine, thus affording an explanation of the apparent anomaly referred to above that plants so closely related as to be placed in the same natural order Solanaceae may produce what at first sight appear to be totally different types of alkaloids, viz. hyoscyamine and nicotine.

There has thus been produced on the chemical side a promising view, supported by experimental evidence, of the processes by which even the most complex alkaloids known could originate in plants, and it now remains for plant physiologists and bio-

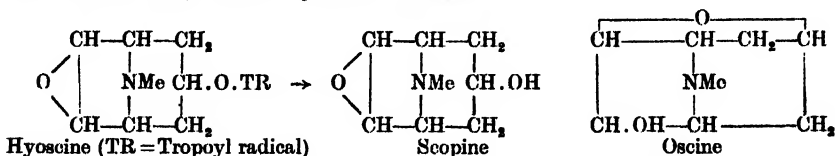
chemists to take up the subject from the biological side and prove or disprove the validity of Robinson's suggestions.

There is one other problem in connection with the bio-chemistry of the alkaloids, which must be referred to. The importance of alkaloids to man is obvious but their value to the plants which produce them is not so clear. Three views have been held as to their function in plants:

- (a) that they are produced as protective agents,
- (b) that they are plastic products destined for further use by the plant,
- (c) that they are merely accidental products of metabolism, that is, waste products.

The first view has all the appearance of having been put forward without serious consideration. If toxic constituents protect plants, animals must be aware that such plants are dangerous and avoid them. There is however no evidence that this happens. Scarcely a year passes in which cattle are not poisoned in England by eating branches of the yew tree, and in countries like Australia, South Africa, and New Zealand where toxic plants have not been largely eliminated from the pastures by generations of careful farming, as they have in England, such plants cause serious annual losses to the pastoralist.

No experimental evidence of great value has been put forward for the view that alkaloids serve as plastic products, in the sense that they are used up by the plant liberating stored energy and being reduced to simple degradation products, but the sounder part of this view, suggested by Bayliss, that alkaloids may serve as stimulants of some kind has never been tested experimentally. It has been applied, with more reason perhaps, by Armstrong to certain of the glucosides, and it should not be overlooked that more alkaloids may occur in plants in the form of glucosides than is at present suspected. At first sight the fact that alkaloids are relatively stable and not highly reactive substances under the conditions obtaining in plants, whereas those glucosides which Armstrong regards as means of storing stimulants, produce on hydrolysis highly reactive substances, discounts the view that alkaloids can fulfil such a function, but it is interesting in this connection to remember that Willstätter has shown recently that the alkaloid hyoscyne when hydrolysed by the pancreatic lipase yields the labile substance, scopoline, which very readily passes into the stable substance oscine long familiar as the hydrolytic product of hyoscyne, when the hydrolysis is brought about by acids or alkalis.



However that may be, the bulk of alkaloid found in any particular plant appears normally to behave as would be expected of a product formed accidentally and without ulterior purpose. It is typical of reactions with organic materials, whether produced by chemical or biological agency, that they are rarely complete or proceed wholly in one direction, and it is to be expected that in the synthesis of proteins from simple amino-acids some by-products will be formed, and that in the degradation of proteins reactions will occur between the amino-acids produced and other substances present in the plant at the same time. When metabolism is active the quantity of

these by-products formed is certain to increase just as the dump-heaps of a blast furnace grow with increased output of metal. It is often carelessly argued that alkaloids, tannins and other normal secondary constituents of plants must be plastic products because their amount is greatest when metabolism is most active but the fact is that real plastic products should be difficult to find when metabolism is most active, because they must be constantly used up and it is only when metabolism slackens that they should begin to accumulate. Perhaps the most convincing piece of work so far accomplished bearing on the function of alkaloids in plants was published by Dr H. E. Annett in India in 1921. He showed quite clearly that in the opium poppy, where the alkaloid-yielding latex is found in the walls of the unripe capsule, that neither the application of nitrogenous manures nor any change in environment affected the proportion of morphine found in the latex, though the quantity of latex formed might be so influenced. In a well-regulated factory one would expect the proportion of waste product to remain constant and the total quantity to increase or diminish as the environment was favourable or unfavourable to factory operations.

Annett also found that in lancing capsules for opium the whole of the morphine was removed in the first few lancements, later lancements yielding latex free from morphine. Further, the seed produced from lanced capsules was as good in quality as that formed from unlanced capsules, so that the latex does not appear to fulfil any special function in connection with the seed during the ripening of the capsule. In addition to special evidence of this kind, the general conditions under which alkaloids are produced and stored in plants, support the view that they are waste products.

Alkaloids are not as a rule found in quantity in seats of active metabolism, such as the leaves, except in short-lived plants where other means of storage are not readily available and even in these cases they are eventually transported to the rinds of the fruits as in the opium poppy, to the coats of the seeds as in some of the solanaceous plants or to the roots as in biennial plants. In perennial plants the usual source of alkaloids is the bark or the seeds, and in the latter, for example in *Strychnos*, strychnine and brucine are found mainly in the seed coats, which are thrown off and the alkaloids lost when the seed germinates.

### III. THE MEDICAL ASPECTS OF THE ALKALOIDS.

By J. W. TREVAN.

(*The Wellcome Physiological Research Laboratories, Beckenham, Kent.*)

THE number of alkaloids which are used in medicine is small compared with the number known. Out of some 800 alkaloids described in Henry's *Plant Alkaloids* only comparatively few have a certain place in therapeutics. There have been, of course, claims that a very large number of extracts containing alkaloids have valuable therapeutic effects, but the number of alkaloids for which there is both scientific evidence from the laboratory, and a consensus of reasonable clinical opinion that they are valuable is about 27. There are many preparations which the increase of therapeutic scepticism in the latter part of the nineteenth century swept out of use, but I think the alkaloids enumerated below probably will be retained in scientific medicine for some time to come. Two indeed have only recently been introduced into general use—sparteine and ephedrine—and I think it is likely that new alkaloids or new uses

for old alkaloids will increase the list still further. The scientific evaluation of the therapeutic properties of a drug on man is an operation of extreme difficulty, much more difficult than its preliminary examination in the laboratory.

Alkaloids used in medicine may be divided into two groups, one producing specific effects on parasites infesting the body, the other producing physiological changes in the body which counteract the disturbances of function set up by disease of rendering possible certain therapeutic procedures involving interference with body functions. In the first group are the drugs given in Table I.

Table I.  
Alkaloids having an antiparasitic action.

Alkaloid	Use
Quinine group	Destruction of malarial parasites
Emetine	Destruction of <i>Amoeba histolytica</i> (dysentery)
Conessine	Destruction of <i>Amoeba histolytica</i> (dysentery)
Pelletierine	Anthelmintic
Arecoline	Anthelmintic.

There are a few other alkaloids to which antiparasitic properties have been attributed but the evidence is not conclusive.

The alkaloids of the second group can be subdivided according to their site of action.

#### (1) DRUGS USED FOR THEIR ACTION ON THE CENTRAL NERVOUS SYSTEM.

##### A. *Depressants.*

1. *Morphine* is the oldest member of this group. It produces its principal effect in the body by diminishing the activity of the cerebrum. It is probably still the most valuable drug for this purpose. Nothing approaching the effect of morphine is produced by any of the modern synthetic hypnotics which pour out in a vast spate year by year from Continental chemical laboratories. The sleep produced by morphine is a nearer approach to natural sleep than any other drug produces. After taking the ordinary hypnotic the patient generally awakes with a headache comparable to that "morning after the night before" sensation which succeeds the sleep produced by over-indulgence in alcohol—which of course is the physiological type of the synthetic hypnotics. This unpleasant effect is absent after morphine and probably because of this very fact morphine is more prone to give rise to the drug habit. I feel that it is illogical to suppose that by any synthesis a drug will be produced equivalent to morphine in therapeutic effect but not prone to give rise to the drug habit. After all the foundation of the drug habit must really depend on the efficacy of the physiological effect produced. The morphinomaniac started his habit because of the therapeutic effect of the drug. Morphine also has a special action where sleeplessness is produced by pain. Much larger doses of the synthetic hypnotics have to be given where there is any great pain. This suggests that morphine has a special action on the ganglia in the base of the cerebrum which are specially concerned with the reception of sensory impulses, and indeed there is evidence from the reaction of the cat that the highest centres in the cerebrum are stimulated by morphine in small doses. Pain at any rate disappears before consciousness is affected when morphine is

administered, whereas the highest part of the cerebrum is put out of action first by hypnotics. Morphine and its derivative heroin are also valuable for their power of diminishing cough by depressing reflex excitability.

2. *Hyoscine* is also used for its depressant effect on the cerebrum. It is used in acute mania. Morphine is not much good in this connection—which is evidence in favour of the suggestion outlined in the last paragraph as to the action of morphine on the cerebrum. Hyoscine has the disadvantage of having certain peripheral effects on the pupil and the secretory glands similar to that of atropine. The peripheral action of atropine is due almost entirely to the laevo-hyoscyamine which it contains. The optical isomer dextro-hyoscyamine, the other constituent of atropine, has no such peripheral action and we are trying it at the moment as a substitute for hyoscine, but the results are not complete.

#### B. *Stimulants.*

The most important alkaloid used for stimulating the central nervous system generally is *strychnine*, which acts on all parts of the central nervous system, increasing the rapidity with which the organism responds to sensory stimuli and diminishing the latent period of reaction. It is almost confined to uses as a general tonic.

One alkaloid has recently found a place in therapeutics because of its more or less selective activity on the part of the central nervous system which is responsible for the regulation of respiration, namely *lobeline*, which stimulates respiration in a very striking fashion.

*Apomorphine* stimulates the vomiting centre.

*Caffeine* also has a stimulating effect on the cerebrum. I have seen acute mania develop as a result of caffeine poisoning, and we all know its effect in the form of tea.

### (2) DRUGS ACTING ON THE PERIPHERAL NERVOUS SYSTEM.

#### A. *On the involuntary nervous system.*

The alimentary tract, the vascular system, the secreting glands, the sweat glands, the muscle of the eye and various other structures are under the control of two sets of nerve fibres which in general have opposing effects on these structures. One set of these fibres goes by the name of the *sympathetic system*, and the other by the name of the *autonomic nerve system*.

##### (a) *Drugs acting on the sympathetic system.*

*Stimulants.* The body itself produces an alkaloid which is concerned with the stimulation of the sympathetic system, namely *adrenalin*. There is only one alkaloid which has found extensive application in therapeutics for its effect on the central and sympathetic nervous system, and that is *ephedrine*, which has an action very similar to that of adrenalin. Its chemical formula is somewhat similar (see Fig. 1). It acts just like adrenalin by increasing the pulse rate, increasing the extent of the contraction of the heart, raising the blood pressure by causing contraction of the smooth muscles of the walls of the arterioles and it dilates the bronchioles. It is much more stable than adrenalin because of the absence of hydroxyl in the phenyl group. Larger doses have to be used of it than of adrenalin, but it can be administered by mouth whereas adrenalin is almost without action when taken by mouth probably because it is destroyed before it is absorbed. The drug comes from a species of *Ephedra* found in China and records of its use are said to be in existence dating from 5000 years ago.

It seems likely that this drug will find great usefulness in the treatment of asthma, the immediate cause of which is a spasmodic action of the bronchioles. An adequate

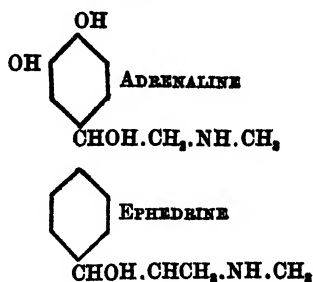


Fig. 1.

dose of ephedrine given by mouth relieves the condition in a few minutes in most cases. Other drugs which stimulate the sympathetic that are found in plants are *hordenine* and *tyramine* but they are very little used in therapeutics.

(b) *Drugs acting on the autonomic system.*

The belladonna group of alkaloids are most important in this connection. *Atropine* is the most important drug. It is made up as already stated of equal quantities of dextro- and laevo-hyoscyamine of which the laevo- is the most active on the autonomic system. It is used principally for dilating the pupil and paralysing the accommodation of the eye, as for instance, when the oculist wishes to examine the retina or measure the optical constants of the refractive media of the eye. It has been to some extent superseded by a synthetic product *homatropine* but its use is still necessary where prolonged action is required or for children. It is also used in relaxing the bronchioles in asthma and bronchitis by paralysing the constrictor fibres which are supplied by the autonomic system. Children are very tolerant of the drug. It has occasional uses in paralysing the nerve endings of the secretory glands and is used as a test in typhoid fever in which an injection of atropine fails to cause a rise in pulse rate as in the normal human. The autonomic fibres which supply the heart through the vagus nerves are continually in activity in the normal animal, keeping a pulse rate lower than it had otherwise been. Atropine paralyses these nerves and so increases the pulse rate. In typhoid fever normal activity of the vagus is in abeyance. The vagus nerve also supplies the intestine so that the paralytic effect of atropine makes it good for colic.

*Physostigmine* stimulates the autonomic nerve endings, causing contraction of the pupil and exaggeration of the movements of the bowel. Its uses in medicine are to counteract the effect of atropine after an optical examination and to stimulate a paralysed bowel to contract after surgical operations.

*Pilocarpine* is the other alkaloid which is used for its effect on the autonomic system. It is principally used for producing sweating in kidney disease.

B. *Drugs that act on the sensory nerve endings.*

*Aconite* has a reputation for producing anaesthesia when applied in the form of an ointment to the skin. It is a highly dangerous drug which is going out of use.

The most important alkaloid which acts on the sensory nerve endings is of course

*cocaine*. It is still the best local anaesthetic available for ophthalmic purposes and for the nose and throat. It has the advantage over all the local anaesthetics so far proposed as substitutes, of causing constriction of the pupil and constriction of small blood vessels both of which properties make it indispensable for use in ophthalmic work. The various substitutes synthesised in the laboratory are displacing it in other fields, but the eye surgeon still depends on it.

### (3) DRUGS ACTING ON MUSCLE.

#### A. *Heart muscle.*

*Caffeine* is used as a cardiac stimulant but not extensively.

*Quinidine* and *sparteine* are used for their depressant action on the heart muscle. There is a clinical condition known as auricular flutter, the principal symptom of which is a very rapid pulse rate and consequent embarrassment of the circulation. The administration of either of these drugs reduces the pulse rate abruptly to normal by an action which has been worked out in detail by Sir Thomas Lewis and his colleagues.

#### B. *Involuntary muscle.*

The work of Macht has shown that *papaverine* inhibits the contraction of smooth muscle. The discovery of this property has led to the use of this alkaloid in the various forms of colic which are produced by spasmodic contractions of organs containing smooth muscles.

There are a certain number of alkaloids which are used for their stimulating effect on the muscle of the uterus namely *ergotoxin* (and the closely allied *ergotamine*) which is used to produce contraction of the uterus after labour, and *hydrastine* and *colarnine* which are used to prevent excessive menstrual haemorrhage.

The alkaloids used to stimulate the kidney to secrete are *caffeine* and *theobromine* principally, and they probably produce their effects by acting on the blood vessels.

The majority of alkaloids that are used in medicine are therefore used for their action in interfering with the normal mechanism of the body. Such a method of treatment is perhaps to be deprecated. It is rather like putting sand into a clock to regulate its time-keeping properties when the pendulum has dropped off. Unfortunately we cannot always find the missing pendulum when the body goes wrong and we have to do the best we can. It is extraordinary, and, I suppose, characteristic of living things how with a little assistance the body can get along with one or more organs permanently damaged. There is one interesting recent example of the condition in which the missing link which gives rise to disturbances of function has been prepared in a form in which it can be administered—the missing pendulum has been found. I refer to the condition of excessive menstrual haemorrhage. It is becoming fairly clear that this is due to a deficiency in a hormone which can be prepared from the ovaries of cattle. The excuse for retaining *hydrastine* in our list should therefore disappear, and the hope of the physician is that with the progress of knowledge more discoveries similar to this will render the use of alkaloids and other remedies for purely symptomatic treatment less and less necessary. With regard to the drugs in Table I the ground for their use is a much more logical one. Their action is directly on the exciting cause of a disease, and it seems possible that additions to the therapeutic armament of a physician will be members of this group rather than of the other. The discovery of new alkaloids with this type of action is of course much more



difficult than that of alkaloids in the other group. We may however take some encouragement from the fact that it is not many years since it was found that Bilharziosis, which is such a plague in Egypt, can be effectively treated by this drug. With the characteristic exasperation of therapeutic research, another specific for this disease, namely tartar-emetic, was discovered almost at the same time.

#### IV. GENERAL DISCUSSION.

CHAIRMAN: One of the questions raised by Dr Henry was the reason for these alkaloids in plants, and he mentioned the suggestion, though I do not think he proved it, that they might be protective. Plants run great risk from insects, and I think it can be said with certainty that the presence of alkaloids is no protection. I remember how greatly the seed and seed capsules of a poppy in Ceylon were attacked by a butterfly which ate them, and in this country the Hemlock has a moth that eats inside it. There is not much evidence that alkaloids are a protection.

One question I would like to ask is whether you (Dr Henry) think there is any prospect of replacing the plant as a maker of alkaloid. In my business, as an Economic Entomologist, we are very dependent on nicotine as an insecticide, but getting it from the tobacco plant is expensive, and if we could have nicotine replaced commercially, we think perhaps it would make a difference.

Dr PETHYBRIDGE: I should like to know if it holds good in most cases that manuring has no effect on the quantity of alkaloid developed. I remember trying to grow tobacco in Ireland, which could be smoked, and in our first efforts the proportion of nicotine was about something like 14 per cent., and the smoking of it was perfectly vile. We were told that the reason was that it had had too much nitrogenous manure, and certainly the fact was proved.

Mr HOWES: I should like to ask Dr Trevan why it is that any native races who make use of arrow poisons can eat the fish without fear of consequences; they use poisoned arrows for game and eat the flesh. Surely these things have alkaloids in them. Is it merely distributed all over the animal and dispersed in small quantities?

CHAIRMAN: One of the things which attracts one about all the alkaloids is how extraordinarily specific they are in their action. In the use of alkaloids as insecticides, it is found that one will be extraordinarily toxic, and a closely related one will have no action whatever. I assume that nicotine is present as nicotine in growing leaves of the tobacco plant, but in spite of that there are a large number of caterpillars which will live and flourish on these leaves, whereas if one gets the nicotine out and puts it on the outside, the caterpillar will eat it and die. It may be that the nicotine is not present in the plant but is produced by fermentation?

Dr HENRY: With regard to the synthesis of alkaloids, it is very unsatisfactory for a chemist to have to confess that although we have been at work on these things for a long time, there is not, at the present time, any case in which a natural alkaloid has been replaced commercially by its synthetic equivalent. The nearest approach is cocaine, which has been developed by Robinson's process, and sparteine has been put on the market, which is not a very good substitute. In the special case of nicotine, I do not think very much attention has been given to that, but it is a more promising

case. I think it should be possible to produce something, but what is proved is, that a good deal of work is needed. I think it is quite likely that if quinine were synthesised to-morrow we should be unable to put it on the market in competition with cinchona.

With regard to manuring, the only effect of manuring is to increase the yield of plants and to increase the amount of alkaloid in that way, but the quantity of alkaloid in a plant is not raised. The case in Ireland was not understood and has never been explained. It merely emphasises the necessity of more work being done on that side.

As to the form in which nicotine exists, what the Chairman suggests is quite likely. The method by which alkaloids are got out of the plant might destroy glucosides, and you could have it in some form which would not be toxic, but that is a point which wants investigating.

Dr TREVAN: Both questions are difficult. As regards poisons—take for instance the arrow poison—the most important types are poisons which contain glucosides of a digitaric type. To begin with, the amount they get is very small, and though enough to kill the animal is nothing like enough to kill a man. You can kill a rabbit with less than a therapeutic dose. That is one way in which I think the native who uses arrow poison escapes. The other is that by the method of injection of poison under the skin the dose to kill an animal is considerably less than that taken by mouth. Another suggestion that has been raised, though I do not think there is any good evidence for it, has been advanced in the case of fish poison; it is said that the toxin accumulates in the brain and natives are very careful not to use the cerebrum (?).

As regards the specificity of alkaloids, that is a long question, and all I know is that it is a most amazing thing that two substances quite similar in structure put into an animal produce different results, whilst two unrelated substances may have quite similar physiological actions.

## JOINT DISCUSSION ON THE CONTROL OF PLANT DISEASES

OPENING ADDRESSES in the joint discussion between Sections 'K' (Botany) and 'M' (Agriculture) on "The Control of Plant Diseases" held in the rooms of Section K at the meeting of the British Association for the Advancement of Science, Leeds, September 2nd, 1927. In the chair Professor F. E. FRITSCH, President of Section K.

I. Opening Speaker for Botany—Mrs N. L. ALCOCK, F.L.S., *Plant Pathologist to the Board of Agriculture for Scotland, Edinburgh.*

II. Opening Speaker for Agriculture—Dr WILLIAM B. BRIERLEY, F.L.S., *Head of the Department of Mycology in the Rothamsted Experimental Station, Harpenden, Herts.*

### I. BOTANY.

#### A. *Author's Abstract.*

- (1) *A short review of past measures in the control of Plant Diseases in Great Britain and how far successful.*

The rise of American gooseberry mildew. Prof. Salmon's warning against American gooseberry mildew and wart disease. Prof. Somerville and white pine blister rust. The saving of the situation as regards wart disease by the discovery of the immune varieties. Action that succeeded—methods that have not succeeded.

- (2) *The present point of view and present administration of control. The essential factors for success in control are:*

- (a) The convinced and willing co-operation of growers. This implies their share in framing legislation.

To attain this end clearness in exposition and ability to see the point of view of the grower and realisation of the business value of all control measures must go hand in hand with scientific efficiency in administration.

- (b) Accurate scientific knowledge on the part of those administering control.
- (c) Moderation in policy when framing rules and regulations for ordinary occasions, and brevity and conciseness in all exposition of such rules.
- (d) Drastic measures for extraordinary occasions.

- (3) *Some questions for the future.*

Plant disease control in *Agriculture*.

Margin of profit here small. Methods must be (a) certain, (b) easy, (c) cheap. Examples—Bunt and Formalin.

*Seed-borne Diseases.*

Some 70 common diseases carried on seeds. Research needed on treatment.

*Horticulture.*

Plant disease control more promising in horticulture. Margin of profit higher. More labour available. Plant sanitation essential. Prevention better than cure.

*Forestry.*

Prevention of disease in nurseries and inspection and control of importation.

*B. Opening Address.*

THE CONTROL OF PLANT DISEASES.

By N. L. ALCOCK, F.L.S.

*(Plant Pathologist to the Board of Agriculture for Scotland.)*

"Be ye ashamed oh ye husbandmen; howl oh ye vinedressers, for the wheat and for the barley: because the harvest of the field is perished. I have smitten you with blasting and mildew."

Joel i. 11 and Amos iv. 9.

The idea that disease of men, of cattle and of plants was a direct act of punishment from the Deity has gradually waned. The view that diseases of men were preventable came first. Veterinary Science has slowly developed and is beginning to have the recognition that is its due, and the youngest scion of medicine, Plant Pathology, is now standing on unsteady feet looking out at a rather unfriendly and sceptical world. Folk still waver between hanging small pieces of looking-glass over the potatoes at the new moon and spraying with Bordeaux Mixture. Ignorance of the nature of plant disease is exemplified by the letter, among the files of the Ministry, saying the writer knew Wart Disease of potatoes was infectious because it had come out all over the baby! But the ignorance of the otherwise highly educated on the subject of plant disease is more troublesome and possibly more profound than that of the peasant.

Continual steady losses go on and it is only by a series of serious attacks that any conviction of their importance is brought home. The first real recognition of plant disease came with the American Gooseberry Mildew. There have always been far-seeing men who saw the importance of disease. Prof. Salmon of Wye in 1905, while the Gooseberry Mildew was still no further than Ireland, gave a clear warning of the necessity for the legislative control of both American Gooseberry Mildew and the potato Wart Disease. Another striking warning, given when the importance of plant disease was not so generally recognised, was that of Prof. Somerville in 1909 (*Quarterly Journal of Forestry*, III, 233). He says, speaking of *Cronartium ribicola*, white pine blister rust, "the disease... is said to be absolutely unknown in America.... But it is to be feared that the day is not far distant when it will gain a footing in North America, and if it spreads there, as it has done in Europe, the loss that will result can only be described as appalling." I believe the loss to-day is reckoned at millions of pounds.

## 546 *Joint Discussion on the Control of Plant Diseases*

But neither the gooseberry disease, which after all attacked a non-essential food, nor the white pine blister rust whose ravages occurred beyond the Atlantic, really awoke England. The first real awakening came with Wart Disease, which though described as a new disease of the potato in England by Prof. Potter and by Mr Massee, in 1902, was not recognised as a danger till some years later.

These two diseases brought legislation. The gooseberry mildew legislation was a feeling in the dark, a stumbling after rules that should help, and it did not always attain the end sought. Gradually experience taught the necessary restraint and moderation, and the amended legislation met the requirement and also the disease for some reason lessened gradually. Let us say because of the legislation, but probably an ebb and flow as yet not understood, occurs in all these troubles, as in human infectious diseases.

Prof. Salmon again spoke in warning of the trouble Wart Disease would bring, but little was heard of Wart Disease for a few years and no very drastic measures were taken. But the disease spread and the American Gooseberry Mildew flourished, and in 1907, another landmark in control appeared with the Destructive Insects and Diseases Act of 1907, an enlargement of that of 1877, which dealt with the Colorado beetle. This was really the initial stage of legislation for Plant Disease Control and brought the whole subject into prominence. The institution of Inspectors to administer the control and Mycologists and Entomologists to recognise and teach others to recognise the pests, was bound to follow and did so.

A third landmark emerged in 1909. This was the discovery that certain varieties of potatoes were immune to Wart Disease. The work resulting in this discovery was carried on over a number of years, and with it the names of Mr Gough and Mr Snell will always be associated. No one knows yet why certain potatoes are immune to this disease, but this fact has stood out for the last 15 years in the annals of Plant Disease Control. So far the immunity has not broken down to any great extent. There are hints in some of Mr Anderson's new work of the possibility of so virulent an infection that this immunity may break down. But we can still say, to quote Dr Pethybridge's short and cogent speech on control, "Thank God for the immune varieties." The Geneticists have helped much, once the discovery was made. Immunity appears to be a Mendelian character and therefore breeders can breed towards immunity with some chance of success, although every new variety has to be carefully tested. Plants that do not take disease are, of course, the ideal and there is plenty of work for the Geneticist in producing plants of all kinds that produce acceptable fruit abundantly, mature quickly, are hardy and resist disease. Till that Millennium we must continue to try to control disease and the value of our attempts to control disease will depend on the simplicity and possibility of the methods we put forward and the clearness with which the knowledge is placed before the grower.

### *The Phytopathological Service in England.*

The service consists of two main sections:

A. *The Control or Official Section.* This consists of:

- (a) the *Pathological staff* at the Pathological Laboratory of the Ministry of Agriculture.

*Entomologist* (Mr Fryer). *Mycologist* (Dr Pethybridge). Assistant Entomologist and Assistant in Entomology. Three assistants in Mycology.

- (b) An administrative unit forming part of the *Horticulture Division* in Ministry of Agriculture, Whitehall, i.e. that part of the Horticulture Branch administering the Destructive Insects and Pests Acts.
- (c) *The Ministry's Inspectorate*, about 30 of whom have special qualifications in regard to Plant Pests and Diseases, though not entirely employed on this work. (All have been specially trained in a short course at the Pathological Laboratory.)

*B. The Advisory and Research Sections.*

- (a) *The Corps of Advisers*, consisting of an entomologist and a mycologist in each agricultural province. Fourteen in number, i.e. 28 advisers.
- (b) *The Research Workers* (about 20 or more) in both Entomology and Mycology. These are at Rothamsted Experimental Station, and also attached to Long Ashton Fruit Station, Bristol, The Imperial College of Science, London, The Fruit Station at East Malling, Lee Valley Station, Cheshunt and the London School of Tropical Medicine (Eelworm work) and elsewhere.

So much for the personnel—the essential factors for success in the control of plant disease are several, but the most important one of all is the convinced and willing co-operation of the grower. This implies his share in framing legislation, his representation in some form of advisory council. Unless the grower really works hand in hand with the Government official, no effective step can be taken. In many cases, the fault lies rather with the scientist than with the grower. The man who is making a living at a very intricate and difficult profession, who has probably been many years at it and often follows his father before him, is usually a shrewd and capable man. In Scotland the level of intelligence and education among the growers is higher than in England. His interest in a new way of fighting disease can always be aroused. But the effective action to follow this stimulus must be founded on sane principles. The business value of the control measure must never be forgotten. The value of the labour, at the time that it is suggested it should be employed, must always be taken into consideration. The value of the materials and use of machinery may exceed the narrow margin of profit in agricultural operations. The cost of the remedy must not exceed the value of the crop. The grower will work with us if we give him the chance. Never tell him to do anything that, in all probability, he will not do, that is too difficult, too complicated, too troublesome. Laws that are not carried out bring the law into disrepute and teach disregard of the law.

Secondly, the knowledge on the part of those administering control must be accurate and wide and above all practical. For instance, in some parts of Scotland spraying gooseberries for American Gooseberry Mildew with plain washing soda is cheap and effective. In other parts of Scotland this spray will burn the bushes badly and destroy the hope of a crop. If you advise a control measure that does harm you may wait long for the trust of the grower to return.

Thirdly, as to details of method. Familiar measures should be used. A table-spoon =  $\frac{1}{2}$  oz., 20 oz. = 1 pint. For instance, the common 2-oz. tobacco tin will hold 4 oz. of liquid. Therefore a 2-oz. tobacco tin three-quarters full will, when poured into a gallon of water make a 2 per cent. solution, and both gallons and

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2-oz. tobacco tins are well known. Both cubic centimetres and percentages should be avoided. In all control, common sense must continually be used.

Clearness in exposition is most essential. Not every practical scientist has the gift of making the facts found out by research clear so that "he who runs may read." But obviously this must be done and rules, orders, leaflets should all be in plain, simple English. As to our leaflets, I have often thought that for those for whom they were meant, a leaflet of one page with a good picture, and a good naked eye description, and the best single method of control is the ideal.

Moderation in policy when framing rules and regulations for ordinary occasions, and again absolute clearness in exposition of such rules, is necessary.

But, now and again, on the extraordinary occasion, the most drastic measure will be the wisest. When a disease appears for the first time in a country and has not spread beyond one or two fields and gardens, could all the contents of the garden, all the crop of that field be put on a bonfire much might be saved. Total destruction of all the disease and all possibly infected material is needed.

### *Seed-Borne Diseases.*

A question that should be investigated and is likely to be discussed more in the near future, is that of the common seed-borne diseases. Many seeds carry loose spores on them (e.g. wheat-bearing bunt spores); many have a resting mycelium (e.g. flax seed with *Colletotrichum linicolum*) and many bear the fructifications of a fungus disease (e.g. sugar-beet seed carries both *Phoma betae* pycnidia and the sori of the rust *Uromyces betae*). *Phoma betae* produces black leg, an exceedingly troublesome disease of seedling beet. There are at least 70 common diseases carried on seed.

Research is needed, especially to find more easy and efficient methods of dealing with these diseases, such as the formalin method for bunt and the hydrogen peroxide method for celery seed and celery leaf spot.

### *Plant Disease Control in Agriculture.*

We have not been faced by the appalling epidemics of some lands. "Fortunately or unfortunately Great Britain has never had a disastrous plant disease epidemic," says Dr Boyce. In the last ten years America says they have lost £100,000,000 through *Puccinia graminis* on wheat alone. But the constant steady toll taken in Great Britain is not noticed, and each year amounts to a very large total. We lose by preventible plant disease, tens of thousands of pounds.

In agriculture the margin of profit is small, the labour scanty.

Methods of control in agriculture must be (a) certain, (b) easy, (c) cheap, (d) safe. A good example is the formalin method of treating bunt. A bad example of a method that is neither easy nor safe is any of the dusting processes, where the dust is poisonous and the operator advised to wear a gas mask.

But the greater field for control in Plant Disease lies in Horticulture. The margin of profit is higher, more labour is available, more good can be done. There is no lack of interest on the part of the fruit grower, at any rate on the part of the good English grower and all the Scottish growers! He is more anxious that his diseases should be overcome than any scientist can be for him. The question of preventive medicine is apt to be overlooked. It is much better to avoid disease than to overcome it. Plant sanitation, good clean cultivation, destruction of all diseased plants, and parts of

diseased plants, and indeed constant destruction of all dead branches, clippings, rubbish and refuse by fire, even the extirpation of weeds, are all methods of plant disease control.

The selection of site is very important. Larch in a low damp site will suffer from larch canker and the incidence of canker on apple trees will lessen as an orchard climbs up a slope. Sour soil will increase club-root.

A great advantage is gained by working with a Botanic Garden. Every Plant Pathologist should really be an expert gardener. As this is not possible, he should work within reach of the expert gardener. Of all diseases that plants suffer from, a great many are not mycological at all. The knowledge of plants, their life and their ways that the really experienced gardener has, is more valuable than can be expressed. At Kew, Mr Coutts, the Assistant Curator, used to say he was the fifth member of the Pathological Staff—every Pathological Staff needs such a fifth member.

The Pathological and cultural knowledge available at the Royal Botanic Gardens, either Kew or Edinburgh, will keep the scientist from being lop-sided.

Both for Horticulture and for Forestry, the great importance of nurseries should be recognised. In Horticulture we can continue to look after our orchard or garden throughout the development of the plant, but in forestry the nursery is usually the one opportunity when disease can be eliminated. To be eliminated, it must be recognised—and never sent about from one nursery to another. In Horticulture and in Forestry much has been suffered in the past by diseased plants being brought into the country. Two motives, one good and one bad, are usually responsible. Good, interesting and useful plants are brought from distant countries. Our gardens have been wonderfully enriched by the Chinese plants of recent years and when these importations have been done through a Botanic Garden, little risk is run. The other motive is the passion for cheapness, the hope of a bargain—some of the very cheap importations are not good. They are not new but only cheap. The classic instance of this trouble is the introduction into America of cheap young white pines and the millions of pounds loss that followed the white pine blister rust that came with them.

To avoid disease is better than to control it.

## II. AGRICULTURE.

### A. *Author's Abstract.*

#### (1) *Scope of Discussion.*

#### (2) *Research Aspects.*

##### (A) *Centres of Research.*

- (i) Source, training and supply of men. Financing and staffing of laboratory, field and administrative services.
- (ii) Types of research centres. Gradation from pure to applied work. Universities, research foundations, crop stations, government and extension services, commercial services. Source of monies and type of centre in relation to scope of work, integration and administrative control.



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### (B) *Fields of Research.*

(i) Principles of control. Disease surveys; plant hygiene and sanitation; plant protection; escapement of disease; exclusion of disease. Effectiveness of methods in relation to expense, social, economic and political implications.

(ii) Some primary general issues.

### (3) *Applied Aspects.*

#### (A) *Popularisation of Knowledge.*

Oral instruction, field service, printed matter. Relative values of the several avenues.

#### (B) *Application of Knowledge.*

Liaison mechanisms; official, commercial, institute and extension, *ad hoc*, personal.

Comparative values of the several mechanisms in relation to types of knowledge and practice. Suggestions regarding organisation.

### (4) *Essential problem for discussion is the integration of practice and research.*

## B. *Opening Address.*

## THE CONTROL OF PLANT DISEASES.

BY WILLIAM B. BRIERLEY, D.Sc., F.L.S.

(*Head of the Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

### *Introduction—scope of discussion.*

In opening, on behalf of Section 'M,' a discussion on the "Control of Plant Diseases" I wish to keep the idea of disease in plants as wide as possible, a more inclusive concept than that suggested, perhaps, by Mrs Alcock in her address. As Section 'D' (Zoology) is not officially represented here, I presume that disturbances caused by the attack of insects and other animal parasites, usually included in the sphere of economic entomology, do not, except in so far as the host response is concerned, come within our purview. We are thus left with, on the one hand, what I may call organic troubles resulting from an excess or deficiency in one or more of the factors of the plant's physical environment—light, temperature, humidity, food supply and so forth and, on the other hand, with diseases resulting from contact with certain factors of the plant's biological environment—invasion of host tissues by vegetable parasites such as certain flowering plants, fungi, bacteria, slime-moulds and the viruses—whatever these latter may be. I take the usual American and continental European view that the term "mycology" is a misnomer for "plant pathology" and that our subject, plant diseases, covers the field I have indicated. It seems to me essential for fruitful discussion that this broad concept of plant disease should be maintained throughout.

Our subject may be approached from two sides, one, the acquirement of knowledge, or the research aspect and two, the application of knowledge or the applied aspect. In plant pathology, as in many other sciences, these two aspects are separated by a

broad chasm, that between research and practice or in the present instance between laboratory and field. We are already in possession of a great amount of knowledge which, if only it could be applied, would go far to solve many of our most urgent problems of disease. On the other hand, there are many field problems of vital importance which, as yet, have scarcely come within the field of view of research men. Further, the practical cultivator often has an invaluable and wide ranging fund of empirical knowledge; hardly perhaps knowledge in the organised and formulated sense, so much as intuitive practice and experience which, could it only be organised and formulated, would be of fundamental importance in scientific investigation and entirely re-orientate many of our points of view. The most imperative need to-day is to bridge this chasm and make available to each the knowledge and experience of the other—to integrate practice and research, the bridge being built to the centre from both sides. It is this bridge that I wish to discuss with you in this meeting. I shall consider the two aspects separately and begin first with the research aspects.

#### *Research Aspects.*

Under this heading I shall include the several phases of our subject which deal with the acquirement of knowledge concerning disease in plants. For our immediate purposes there are two main and correlated issues, on the one hand, the scientific workers ranging from the specialist in the research laboratory to the general practitioner in the field, and on the other hand, there are the actual problems of disease which require to be solved. For convenience I shall speak of these as “centres of research” and as “fields of research.”

#### CENTRES OF RESEARCH.

This sub-heading may be still further resolved into its “human aspects” and its “institutional aspects” and I shall consider these in this order.

*Human aspects.* In the past, and still largely to-day in this country, the study of plant disease has been regarded merely as a post-graduate and specialised development of scholastic botany. A student takes the usual degree courses in botany followed perhaps by a few months intensive study of the fungi and perhaps a year’s “training research” on some minor fungus disease and is then turned out on the world as a fledgling plant pathologist. In the earlier simpler days of classification and description this “pure” botanical training of the plant pathologist and the subsidiary dependent status of the science were perhaps not harmful although, personally, I cannot help feeling that the outcome has been a narrower and more scholastic vision than has been desirable. With however the vast importance and wide scope of the science as, patently, it is with us to-day with its roots in botany, physics and chemistry, soil science, horticulture, forestry and general agriculture, I would ask you whether the view still commonly held by academic botanists in this country is any longer tenable. It is rather like a foster hen continuing to mother a turkey. The academic botanist may be right and plant pathology may be his very own wayward child, but the matter seems ripe for discussion. Let me put the issue in certain concrete questions.

In the first place, as a matter of recruiting, is the academic man with his “pure” botanical and inwardly directed vision as suitable for our purposes as the agricultural college man with his more general training and outwardly directed vision? The one is more usually town born and bred, with a townsman’s outlook, and his academic training leaves him usually, with a townsman’s attitude towards the regimented

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growth of plants under commercial conditions of forestry, horticulture or agriculture. The other is more usually country born and bred, with daily experience of practical conditions, with the countryman's outlook and almost intuitive feeling for growing crops, and these things broadened, refined and formulated by his training in an agricultural college. The difference is largely one of mental attitude and outlook, and I would put it to you as a matter for discussion whether we do not need, as plant pathologists, men whose scientific vision is extrovert rather than introvert.

Again, is plant pathology merely a post-graduate specialisation of academic botany? The ordinary botanical student is trained by academic botanists as an academic botanist. Some months additional mycological study in a botanical laboratory or a herbarium does not make him a plant pathologist, it makes him, perhaps an embryo mycologist. A plant pathologist must have a general botanical outlook with considerable knowledge of the structure and genetics of cultivated plants and of their physiology, especially in relation to conditions of climate and soil, storage and transport. He must be intimately acquainted with the bacteria, fungi, etc., and this not so much from their academic aspects but from the standpoint of their activities in the glasshouse, field, plantation, transport waggon and storage shed, as causal agents of disease. He must have a knowledge of commercial conditions and practical processes in horticulture, forestry and agriculture and of practical hygiene and sanitation of cultivated plants in all general aspects which may bear relation to disease. He must have some acquaintance with economic entomology and helminthology and sufficient knowledge of physics and chemistry to develop prophylactic and therapeutic treatments such as spraying, dusting, fumigation and so forth. Finally, he must possess sufficient comprehension of the world situation in agriculture, horticulture and forestry, its geographic and economic implications to understand problems of plant introduction, national and international action relating to embargoes, quarantine and so forth.

I would ask you, is such a science merely a post-graduate aspect of botany for which the ordinary academic training is the all sufficient requisite, or is it better to regard it as a science of independent status, as distinct from botany as, for example, veterinary medicine is from zoology or engineering from mathematics? I do not want you to entertain the wrong idea that I think plant pathology can do without botany or that engineering can do without mathematics, for that is not the case. Botanical science is the rock on which plant pathology is founded and on which it must be developed. The question in my mind is whether the botany of our academic schools is the right kind of rock or whether it is not, in perhaps most scholastic centres, quite unsuitable. The fact of the matter is that, to-day, the plant pathologist, as a professional man analogous with the veterinarian or the medical man, has only in few cases the time and money to obtain his vocational training subsequent to a degree in botany except in the event of the development on a vastly increased scale of the system of Research and Travelling Fellowships, Bursaries, etc., adopted by the Ministry of Agriculture, the Cotton Research Association and certain other bodies. Only the research specialist in one or another aspect of plant pathology like the medical specialist can afford to spend several years gaining experience. To my mind the general practitioner in plant diseases will increasingly need to compromise and to obtain more of his vocational training as a part of his degree training—a degree in plant pathology. Adequate training for such a degree cannot, so far as I can see, be obtained within a pure academic school of botany. We need

for Biology something analogous to what the Colleges of Technology have done in Physics, Chemistry, Engineering, etc. I think the ideal would be a School of Plant Pathology intimately cooperating with Schools of Botany, of Horticulture, of Forestry and of Agriculture, or, if not an independent school, then administered perhaps from the School of Botany but with these contacts as an integral part of its existence. Plant pathology seems to me essentially an independent vocation as autonomous as the medical profession or the veterinary profession. Such a special vocation demands recognition, adequate stipends and provision for a career as obtains, for example, in veterinary or human medicine. A beginning in plant pathology has been made at home in the Agricultural Research Service of the Ministry of Agriculture and promised in the Empire by a visioned colonial agricultural research service, but commercial avenues have, as yet, hardly been thought of in this country or in many of our overseas dominions.

Many of my botanical friends will feel that I have been too drastic in what I have said concerning academic botany but I would beg them to remember that I am opening a discussion on behalf of Section 'M' (agriculture). Further, I have myself passed through the ordinary academic botanical training and may therefore be presumed to have a bias in its favour. On the other hand, my experience is much wider than that of most teachers of botany in that I have not only held posts on a University botanical staff but also on the staffs of Kew and Rothamsted. Throughout, and more especially since the war, I have had considerable experience with students and "practitioners" of botany, of horticulture and of agriculture both at home and abroad and the views I have put forward largely do represent the outcome of this, perhaps unusually wide experience. Certain ideas and points of view in botany have almost acquired the sanctity of the thirty-nine articles and to question orthodoxy is not popular with the botanical Episcopate. It would have been very easy for me to chant "God's in his heaven—Alls right with the world!" of academic botany and plant pathology; to utter pious platitudes about the need of research and about "pure" science so spotless and unsullied, to argue that all that is needed for the perfect plant pathologist *sans peur et sans reproche* is to make quite sure that he gets a thorough grounding in academic botany and that all will then be well, and so forth and so on. These are the simple articles of orthodox faith and if one anoints oneself with vegetable oil of self-righteousness and swims with the current plaudits are assured. It is neither an easy nor a popular position to be an inside critic and to abandon the my-subject-right-or-wrong attitude, but my personal experience is leading me to think that all is not well in the state of academic botany in its relation to plant pathology, and that this sickness is, not improbably, one of the main roots of our trouble in the supply and training of men for the control of plant diseases. As a botanist myself I rather hope I shall be proved wrong but, in any case the question is, I think, meet for discussion, and all I ask is that it be discussed with an open mind and without relation to the vested interests of botany.

I have not lost sight of the fact that unusual men, the leaders, will do unusual work whatever their training, and I rather think that such men will most commonly arise in the "pure" sciences; or of the fact that different aspects of a plant pathological training might be stressed accordingly as the more usual student envisaged a career as a specialist in a research laboratory, as a general practitioner or "medical officer of health" in the field, as a teacher of plant pathology, as a member of a field

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inspectorate or advisory service, as an administrative officer and so forth. These, however, seem to me minor issues when considering the status of plant pathology as a science or in treating in general of the research aspects of our subject.

Let me now pass to my second sub-heading the "Institutional aspects."

*Institutional aspects.* A general consideration of centres in which research on plant disease is carried out shows a very wide range of types of institution or organisation. To illustrate what I mean, I may perhaps use the analogy of a political assembly in, for example, Germany. On the extreme right wing are the scholastic institutions with their die-hard "pure-science" attitude, whilst on the extreme left wing are the commercial laboratories with their purely economic attitude. Ranging from one wing to the other are all the various grades of institutions or organisations containing pathological laboratories or departments. Let me instance certain examples of each main block.

Moving away from the right-wing universities in which the outlook is essentially academic and purist, one comes to many of the great research foundations in which, although "pure research" is carried out, the view-points have a more applied direction. One may instance the Rothamsted Experimental Station in this country, the Biologischen Reichsanstalt für Land-und Forstwirtschaft in Berlin-Dahlem or the Institut für Pflanzenkrankheiten in Bonn-Popplesdorf; the Phytopathological Institute in Wageningen, Holland; the Pusa Research Institute in India, and in the United States of America, the Bureau of Plant Industry at Washington, the Boyce-Thompson Institute for Plant Research and the Tropical Plant Research Foundation.

Passing still more towards the left are what may be called the applied crop stations, although the word "crop" is not so applicable in certain cases as the term "cultivation-area." In this country are the experimental and research stations at Cheshunt, East Malling and Long Ashton; certain agricultural colleges such as that at Wye in Kent, the Royal Horticultural Society's laboratories at Wisley, certain branches of the Department of Scientific and Industrial Research and of certain Industrial Research Associations and so forth. The new Amani Station in Africa and the visioned chain of Empire stations might find their place here along with more special centres such as the Hawaiian Sugar Planters' Station, the rubber stations in Ceylon and the Federated Malay States, the tea stations in India and Ceylon, the famous Dutch stations in Java and Sumatra, the West Indian Cotton Station and perhaps most of the agricultural experimental stations of the United States of America.

Of still more applied outlook are many of the "research-advisory" organisations such as the agricultural advisory officer service and the Imperial Bureau of Mycology in England, or the colonial mycological service overseas; and on the left-hand of this block stand the "advisory-research" organisations such as the Inspectorates and pathological services of the various governments, the extension services of Denmark, the United States of America, etc.

Passing to the extreme left wing one comes to the commercial organisations some of which are acquiring men of established reputation and are obviously destined to play a primary part in applied research of the future. In this country may be instanced the scientific officers of the Mond corporation or, on a small scale, such firms as Mason, Munro, Abol and so on, and in America the Bayer Co., the Niagara Co., the Pont de Nemours Co., the Bowker Chemical Co., etc.

Finally, there are the several journals in all countries which give practical advice or answer enquiries concerning plant disease.

When one surveys the wide scope and entirely uncorrelated nature of these organisations, not only from country to country but even within any one country, one realises how little economy in ideas, in men, time, energy or money there is in the present manner of their existence. One cannot help feeling that there should be, to take only those within the British Empire or an even smaller area, those in this country, much greater ease and freedom of exchange of men and ideas than exists, and much greater coordination of activities. It is imperative, however, that any co-ordination must be achieved without introducing the arid and sterilising influence of red tape, of unenlightened direction or of merely administrative control. With the increasing dependence of many of these organisations on government or public monies some degree of integration of staffs and activities seems inescapable, if only in the interests of economy. It is extremely desirable that we should realise this and take steps to ensure that any coordination shall not be in the interests of economy only, but also in the interests of pathological research and application and the best utilisation of available facilities to liberate the greatest good. Freedom in research does not come from the absence of organisation but is dependent for its very being upon organisation.

Further, if some degree of coordination were brought about in existing pathological organisations it might not only liberate much energy at present locked up in unskilled administration and the almost entire absence of any methods of business economy, so patent in most scientific laboratories, but it might result in the very positive gain of greater financial and practical support for the more right-wing institutions. At present the latter are almost entirely supported on rare endowments, private subscriptions and, ever preponderantly, on public monies but with such co-ordination that the practical man is brought to realise their potentiality for good, it might be possible to tap the "trade-purse" either for particular institutions or particular researches. Many of the primary crop stations are supported almost entirely by voluntary levy by the planters or traders of acreage "cess" or produce "cess," and there would seem no reason why this basis of support should not be extended in many directions.

Again, if only a broad and constructive policy could be applied to the integration of our teaching, research and practical organisations dealing with plant disease and cognate issues, so that right and left wings moved in unison to the one common end, the influence upon both extremes would be most salutary. Many academic schools of botany, for example, seem to live in a little cloistered world of their own far from any sullyng contact with practical realities; a world in which plants exist as herbarium specimens, or pickled fragments in bottles; systematically arranged in botanic gardens or ecologically arranged in salt marshes. The drilled regiments and legions of plants living and dying on farm or plantation seem to have no meaning for them although our very civilisation depends upon such "crop botany." On the other hand, the grower surrounded by square miles of rubber or cocoa, sugar cane or wheat, with disease gaining upon him day by day, is apt to be impatient with the academic scholiast. To an autonomous plant pathology all this would be of little moment for it would work out its own salvation, but to a plant pathology, which, as in this country at present, is dependent on the schools of botany, the matter is one of serious import. What I, as a plant pathologist, wish to see are the schools of botany linked in closest intimacy with agriculture, horticulture and forestry so that botanical students

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realise from the beginning that a cucumber house, a potato field or a rubber plantation are just as much objects of botanical study as the marine algae or an apical meristem.

We need enthusiastic and constructive imagination to integrate our pathological organisations from right wing to left so that the wings shall spread and yet come together in flight, so that the schools of botany shall become streams of inspiration finding their source in the world's demand for healthy crops.

So far I have been discussing the human and institutional aspects of research on plant disease and I shall now turn to what may be called the "fields of research" in plant pathology.

### *Fields of Research.*

I shall not treat this portion of my subject in any detail as it has already been considered by Mrs Alcock in her address. The discussion falls naturally into two portions, one dealing with the general principles of control and the other dealing with specific issues.

*Principles of control.* There may be said to be some five primary avenues in disease control.

Firstly, are disease surveys, in which record is made of the presence and incidence of domestic or foreign diseases. Such work leads to international surveys and knowledge of the geographical and climatic relationships of disease.

Secondly, are all the problems connected with plant hygiene and sanitation, including the elimination of infective centres, the relation of nutritive and other physical conditions to parasitic diseases and to non-parasitic diseases and cultural methods for reducing the incidence of disease.

Thirdly, are the questions concerned with plant protection, such as spraying, dusting, steeping, fumigation, sterilisation, and so forth in all of which the fundamental value of intimate contact of plant pathology with chemistry and physics is becoming more and more evident.

Fourthly, are the problems of disease escapement, which comprise the selection or breeding of escaping, tolerating, resisting or immune varieties, the relation of time of planting and other cultural operations to disease, the questions of rotation and suitability of crop with all their corollaries.

Lastly, are the issues of disease exclusion, questions of plant introduction, of embargoes, and quarantines and legislative action in their domestic, national and international aspects.

It will immediately be clear, if one considers these principal fields of research in relation to the centres of research already indicated, that different types of institution or organisation are better adapted to advance progress in certain fields than in others. This is largely a question of the source of financial support and the facilities and men available. Thus, problems of disease survey and disease exclusion would seem best fitted to government pathological services with their adjuncts. Problems of plant hygiene and sanitation and disease escapement would on the whole seem most suitable for study by agricultural, horticultural and forestry centres. Much work concerning plant protection might perhaps profitably be carried out by the commercial organisations and so forth. But in whatever manner the various issues apportion themselves there are certain fundamentals that underlie all pathological research and application. For example, any control method must be viewed not only in relation to the expense of its application and its commensurate return, but also in relation to its social,

economic and political implications. A method suitable for a highly organised plantation system might be quite unworkable under a system of individual or peasant cultivation. A method which might be adopted successfully by an educated community might fail dismally in an ignorant community or, alternatively, succeed in such a community with a trained executive. Methods suitable for single-crop systems of cultivation are often unsuitable for rotation systems. Methods which may be used successfully in pure-line or clone culture may fail in mixed-population culture. Plant introductions, embargoes and quarantines may need to be modified in relation to free-trade or protectionist policies. And, fundamental to all issues, is the purely economic one of the cash return of the crop, perhaps low prices on a bumper crop, high prices on a small crop, world prices, domestic prices and so forth.

Before leaving this aspect of our discussion let me indicate briefly certain issues of plant pathology which seem to me of primary importance to-day, issues upon which research is most urgently needed.

Firstly, the whole congeries of problems concerned with the relation of meteorological, edaphic and nutritional factors to parasitic disease. At Rothamsted Mr Stoughton and myself are commencing the study of physical and nutritional relationships, both in their fundamental aspects and in direct connection with certain diseases of the cotton plant.

Secondly, the physiological-genetic study of parasitic fungi and bacteria. At Rothamsted, Mr Dickinson, Mr Stoughton and myself are working on these problems.

Thirdly, the epidemiology of plant disease, a subject on which we possess very little accurate knowledge.

Fourthly, the nature of the several kinds of susceptibility, tolerance, resistance or immunity to disease. At Rothamsted Miss Glynne is studying certain aspects of these problems in relation to Wart Disease of potatoes.

Fifthly, the field selection or breeding of resistant and immune varieties in relation, on the one hand, to strains of parasitic organisms and, on the other hand, to geographic and climatic areas.

Sixthly, the whole new world of problems included in virus diseases of plants. This complex of troubles surely forms one of the primary controlling factors in the world's agriculture to-day but, considering the importance of these diseases, deplorably little is being done in their study. One of the main reasons is that so few pathologists are really competent to carry out investigations into these difficult and obscure regions, for it is imperative that such work should be done with a meticulous accuracy and impeccable technique such as have rarely been demanded in previous phytopathological studies, and, also, that it should be done with full cognisance of similar researches into virus diseases of animals and man. At Rothamsted the virus disease investigations are being carried out by Dr Henderson Smith.

Seventhly, the possibilities of the cultural control of disease. This is a much neglected but extremely promising field of research which might give immediate practical returns. Of the possibility of the biological control of fungal and bacterial parasites, analogous to the biological control of insect pests, I am not sanguine.

Eighthly, the whole complex of pathological problems connected with transport, storage and marketing. Such researches are almost an untouched field in this country but have a future of tremendous importance.

Ninthly, the physico-chemical basis of methods of plant protection. At Rotham-



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sted Dr Tattersfield *et al.* are studying these problems in relation to entomology and parallel researches from the fungal and bacterial side are urgently needed.

Tenthly, the practical value of the several grades of embargo, quarantine, etc., and of legislative action in controlling the dissemination of plant pests.

Lastly, the methods of estimation and statistical analysis of the incidence of disease and the losses from disease.

Of course plant pathology is "so full of a number of things" that one could continue listing problems for some considerable time, but, those that I have just suggested, do seem to me to be of such outstanding importance that any addition to our knowledge would very materially increase our prospects of understanding and controlling plant diseases. It may be noted that certain of these problems might be considered of merely theoretical interest. Well! plant pathology has been such a mushroom growth, and so empirical has been its development, that it has had no time to think philosophically or consider its theoretical foundations. Speaking generally and with, as it were, a long distance point of view, I am of opinion that the greatest need of our science to-day is theory and that with an advance in our philosophy will come a real advance in our practice. It is thus that I find myself, a little to my surprise, holding orthodox views as to the need for more fundamental research. The only proviso I would make is that some of the energy devoted to this end should be directed towards synthesis and not, as at present, wholly towards analysis.

So far I have dealt only with the first portion of my subject, the "research aspects," and I now wish to pass to the second portion, the "applied aspects."

### *Applied Aspects.*

Of the vast amount of data, or of knowledge, acquired during passing years by scientific investigation the greater part that reaches publication stage is printed with all due ceremony in some technical journal and so quietly laid to rest. Occasionally the corpse is disinterred and its bones examined but the second burial is usually quite final. Now a fraction of this research has potential practical value but it is rarely or never carried beyond the laboratory stage and so this value is usually lost. The questions I wish to consider with you are, firstly, how the knowledge that we possess written in technical jargon or locked away in scientific journals can be popularised and disseminated and, secondly, how potentially practical researches can be carried forward to a stage of successful application. I shall discuss these aspects in turn under the headings "popularisation of knowledge" and "application of knowledge."

### *Popularisation of Knowledge.*

There are perhaps three main avenues by which knowledge concerning plant disease in its several aspects may be spread among a rural population.

In the first place is what may be called the teaching avenue which commences with the elementary schools, more especially, perhaps, the rural schools. I think we are all orthodox in our demands for far more biological teaching in schools but, where I should perhaps go beyond many, is in my insistence on the fact that, even in school-teaching, disease and death should be regarded as just as much a part of the normal life-cycle of animals and plants as are birth and health. The two form the complete circle and, to leave out one-half, is to falsify every issue for each only has meaning in values of the other. More biological teaching and, what is equally important, sounder biological teaching, in the schools would go far to produce a rural com-

munity with a more understanding mind concerning health and disease, life and death, not only in plants but in animals and man. More advanced and formal teaching concerning plant diseases is given to a moiety of future cultivators in agricultural high schools and colleges, horticultural institutions and so forth. In all of these, problems of disease and death are usually cut off from normal plant functioning as though they were something not mentioned in polite botanical circles. This, I think, is a mistake. An extraneous teaching avenue, which one almost hesitates to mention, is wireless broadcasting, but so many receiving sets are now spread widely over the countryside in all lands, that it is quite obvious that this avenue might become one of the most important not only for general instruction relating to plant disease but of very direct and urgent use in the face of sudden outbreaks or epidemics of disease.

One of the main avenues of popularisation leading to the adult rural community is the formal lecture system—there are lectures organised by government departments or subsidiary services, lectures by educational and research institutions, lectures by growers' organisations of all kinds, lectures *ad hoc*, lectures almost *ad nauseum*. The value to the lecturer, especially if he be a right-wing man, in giving him contact with the practical farmer and grower is undoubted, but, after a considerable experience of lecturing, I am becoming sceptical of much value, save in the most general sense, accruing to the practical man.

Infinitely more important than formal lectures, to my way of thinking, are actual demonstrations, field trials and experiments, if possible carried out locally in the districts or conducted more centrally by agricultural colleges or research stations, and actual demonstrations of laboratory research in progress. Intimate contact of practical man and research man by visits of farmers, horticulturists, and foresters to educational and research centres and much more visiting in the converse direction, would induce a far greater appreciation of scientific research work and go a longer way to the encouragement of an educated adult rural community than any amount of sporadic lecturing. The only essential is that any demonstration of research should not be a perfunctory showing by an unwilling and bored junior, but should partake of the nature of an actual educational experiment by an enthusiastic and skilled demonstrator who can meet the practical man half-way.

A second primary avenue of popularisation of scientific knowledge may, perhaps, be termed the official channel. By this I mean the central official pathological laboratories and field services, the advisory and inspectorate organisations, the smaller local organisations and extension services, the official channels in growers' organisations and so forth. All of these carry on valuable activities and, mostly in respect of particular and local problems, do transmit to a minority audience knowledge concerning plant disease. Personally I do not think too much importance can be attributed to the growers' organisations and, I feel strongly, that their potentialities for the spread of knowledge have not been sufficiently recognised or developed.

A third main avenue of popularisation of knowledge is printed matter. Here are included notices of legislative enactments regarding disease, the excellent leaflets on specific plant diseases issued by the Ministry of Agriculture and the agricultural colleges, the official journal of the Ministry and various year-books. Other countries have developed such official publication to a greater or a less extent. In the United States of America, for example, this avenue is tremendously developed; many of us who have to read the publications sometimes feel that "overdeveloped" might be a truer word. In this country a recent and noteworthy attempt to give the practical man an idea of what is happening in research laboratories financed by the Ministry

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of Agriculture is the volume by Wilkins entitled *Research and the Land*. Records of scientific activity are also attempted by the Royal Agricultural and Royal Horticultural Societies and of more popular nature are such publications as the *Fruit Grower*, *Farm and Field*, the *Gardeners' Chronicle*, etc. A further channel of dissemination which is becoming increasingly important is trade advertisement and propaganda.

Surveying the issues broadly it is very difficult to estimate the value of these different avenues of popularisation. I imagine that no one questions the immense importance of the mental perspective of the rural community towards crop growth, determined initially in the rural schools. After a considerable experience of lecturing to all sorts and conditions of audience I am inclined to think that, as a means of conveying data or knowledge, incidental lecturing to farmers and growers is of comparatively little value, save, as I have already indicated, to the lecturer. The rare man who can strike the imagination of his hearers and arouse enthusiasm in them to take up special questions as a personal interest is, of course, in a different category. One of the most painfully outstanding things in modern science, however, is the rarity of such men. Organised courses under such conditions as those obtaining in the Workers' Educational Association would also, I think, need to be regarded differently and if the rural community could be invaded by a W.E.A. having biological interests this channel might become of extreme value. My experience and opinion regarding the value of printed matter is that, commonly, it is astonishingly less than might have been expected, for the practical man seems often to find it very difficult to translate the published word into terms of personal experience and field practice.

So far as my personal experience goes, by far the best avenue of popularisation is by quite informal discussion with growers, and, above all things, by practical demonstrations where the grower can see things for himself, in the flesh as it were, and make his own judgments in the light of his own knowledge and experience. I feel that I cannot advocate too strongly the value of such intimate and informal contact between the research man and the practical man, and one of the best ways to bring this about seems to me to multiply opportunities of contact by field and laboratory demonstrations, informal discussions and any other way in which the field is brought to the laboratory or the laboratory to the field. I think if energies were directed to this end by the organisations existing on both sides, the spread of scientific knowledge among the adult rural community, and, what is equally important, the spread of practical wisdom among the adult scientific community, would increase rapidly, and we should advance toward that sympathetic and understanding liaison of research and practice which is so urgently to be desired.

Let me now pass to what is the final matter I wish to consider with you, namely, the actual mechanisms of liaison and application.

### *Application of Knowledge.*

The channels by which new processes or new products may be conveyed to the practical man, so that they become an integral part of his practice, are very numerous and varied.

Firstly, there is what may be termed the official channel. In the majority of countries there is a more or less official chain of universities, research stations, government pathological services both central and local, inspectorate and advisory services, extension officials and so forth. Implicit in this organisation is, I suppose, the idea that research coming from the right wing which might have a practical outcome is

taken up by the more left wing services and worked out in its practical or field applications, ultimately finding its way *via* the inspectorate, advisory or extension services into actual practice.

Secondly, there is the commercial channel. On the one hand, research emanating from "private" laboratories or from any published source may be worked out to a commercial issue by the scientific officers of an interested firm and put on the market *via* commercial channels for private profit. On the other hand, a non-profit-making syndicate might be formed to provide facilities for the practical working out of a scientific discovery, to commercialise the product or process and put it on the market in the interests of the further development of the particular issue. Such, for example, was the procedure adopted in the exploitation of the "Adco" manurial process.

Thirdly, there is what may be termed the Institute method. The scientific staffs of crop stations or other similar institutions, not only carry out applied research, but investigate in their own legitimate interests the practical value of any published research, perhaps modifying or carrying on a more theoretical investigation to a practical issue. Such stations are usually conducted in the interests of one or more special crops and the organised growers not only have direct and often predominant representation upon the governing body of the station, but take a very lively and keen personal interest and pride in the work done. Both by intimate personal contact, demonstrations and publications, results of practical value are made known immediately to the growers who put them into actual practice under the advice and often under the personal supervision of the scientific staff. In many countries there are central institutions more or less comparable to the National Institute of Agricultural Botany at Cambridge, and these take a greater or lesser share in the application of scientific knowledge to field practice. Apart from the Wart Disease of potato trials carried out by the N.I.A.B., no pathological work, so far as I am aware, is done under the aegis of this Institute and there seems ample scope for the extension of the activities of such a foundation into the realm of disease.

Fourthly, is what may be called the extension avenue. Except in so far as extension work is carried out in this country by the Ministry's inspectorate and the advisory services this channel which in Denmark, the United States of America and many other countries is so important and vital, is little developed. It is, of course, an avenue of popularisation as much as application but in the U.S.A. where the Extension services are linked, on the one hand, with the experimental stations, and, on the other hand, with the growers' organisations, it is undoubtedly one of the largest factors in actually getting knowledge applied.

Fifthly, is what may be called for want of a better term, the *ad hoc* mechanism. There are already many committees of public, scientific or practical organisations which have been created specially to look after particular issues. Committees of responsible bodies such as the Ministry of Agriculture, the Forestry Commission, the Royal Agricultural, Horticultural or Arboricultural Societies and so forth might well be created *ad hoc*, on need being shown, for the obtaining of facilities for the carrying on of a laboratory research to a practical end, and for ensuring in any feasible way, that the results were made available for successful adoption in practice.

Lastly, is what may be termed the personal channel. In this case the research man himself carries on his work to the practical stage, and then, by his personal effort, sees it through the avenues of propaganda or other mechanism of publicity and finally puts the process or product upon the market, or in some other way ensures its adoption by the grower or interested party. The man who is best for the pure investi-

gational aspect is rarely the man most suited to propagandist issues or one who shows any great acumen in the commercial exploitation of results. I think many of us must know workers of considerable research ability who are becoming, or have become, lost to science, because willingly or not they are following up their research work into the arena of practice. On the other hand, such a method does tend to maintain stricter accuracy in the actual presentation to interested parties of the facts of the case and, further, keeps alive an atmosphere of single-minded purpose and personal enthusiasm which often leads to success.

Of course in discussing any of these mechanisms of application, and this also applies to avenues of popularisation, the several methods I have indicated do not, in actuality, work independently but form a complex in which one aspect or another becomes increasingly emphasised or predominant. Further, the choice of avenue is often, to a large extent, dictated by the source of the knowledge or applied research. With the increasing tendency of commercial organisations to retain their own scientific officers it is obvious that an increasing amount of research or application, deriving from commercial laboratories, will be marketed for profit by the organisations concerned. Again, the avenue depends considerably upon the type of "goods" to be marketed or distributed. Certain products or processes are marketable whilst others are not; thus it is a simple matter to put on the market a new immune variety, a new spray mixture, a new type of machine and so forth, but a difficult thing to make available for successful adoption a new cultural operation, a new planting time or new hygienic methods in which the active and intelligent cooperation of the grower must be enlisted and which he, personally, may need to carry out without any obvious and immediate return. The mechanism of application thus further depends upon the type of community, whether one of centralised plantations or of peasant cultivation, an educated community, or an ignorant one and so forth. Finally, the question of "politics" may be involved, embargoes, quarantines, import regulations, diseases and pests legislation, etc., but as Mrs Alcock has already considered this aspect I shall not discuss it further.

In the field of "application" as in that of "popularisation" I feel that I cannot emphasise too strongly the value of small "crop" or "cultivation area" stations in which there is the most intimate contact between scientific investigator and practical grower. If I may be forgiven an actual example I would instance the Experimental and Research Station, Cheshunt, where work on tomatoes and cucumbers is carried out. I would state, however, as my considered opinion, supported by every shred of my personal experience, that it is most desirable, indeed almost imperative, that such crop stations should be kept small. If it is found that one small research centre in any area is insufficient for the needs of that area or crop then, the original station should not be increased in size, but a second one should be established. In this country, for example, I do not think any more large central stations are required; if money is available the existing ones might be better staffed and equipped. On the other hand, I do not think we can have too many small unit stations, and I look forward to the day when the agricultural, forestry, and other cultivation or crop areas will each contain a smaller Cheshunt or East Malling. What I would like to see are small, simply but adequately equipped laboratories, with if required mobile adjuncts, containing three or at the most four trained men—a plant pathologist, a crop physiologist, an entomologist and a chemist. These men should be essentially field men with a practical outlook and an intuitive "flair" for the growing and regimented crop plant. Their appointments should be conditional on their spending three or four months of each year, or

one year in three or four, carrying out study or investigation in some University or primary research centre like Rothamsted. Their work should be essentially practical research on local problems; not academic work as so frequently is done now but actual frontal assaults on field problems of disease, a large number of which are susceptible to direct empirical attack in the total absence of theoretically desirable knowledge. Further, they should carry out experiments and demonstrations under controlled and under local commercial conditions. I think that the establishment of such units would ensure not only fruitful research on problems of diseases of plants in the field, glasshouse or plantation, but it would multiply opportunity for extensive popularisation of such knowledge, and give prospect of the successful adoption by the practical man of prophylactic and therapeutic treatments. It would lead directly to the integration of practice and research which is the great desideratum.

If one looks over the whole field in the light of one's own experience, both in this and other countries, one cannot help feeling that there exists a real need to-day for a wide survey, somewhat along the lines I have suggested in this address, of the problems of research and practice in relation to disease in cultivated crops. But such a survey needs a broad and constructive vision, that would transcend vested scientific interests, and a courage that would not hesitate to suggest building here and scrapping there in the interests solely of progress towards plant health. If real advance is to be made, it is essential that all the primary issues of pathological research shall be covered economically and thoroughly by men of sound training, of practical outlook and with direct knowledge of commercial conditions. And, secondly, it is essential that relevant information shall be made available to the practical man in such a way that it shall be adopted, translated into field terms and successfully applied. The greatest need in the control of plant diseases is to integrate research and practice and to bridge the gulf from both sides.

As many, perhaps most in this audience are teachers or senior students of botany, agriculture or plant pathology, I would like to say, in concluding, what to my mind is the most fundamental thing of all. I would plead that in our studies we break away from the arid and sterilising grip of unimaginative empiricism in which our sciences are held to-day, that we abandon the dreary and paralysing formalism of "pure" academic tradition and cloistered scholasticism and, whilst retaining all that has value in "pure" science, mould this science fundamentally and boldly to the needs of the new era and the new human issues. That we go out into the world and seek our inspiration in the reality of the growing plant and largely in the plant grown to satisfy human need, for only so shall we keep in tune with the progress of our times and minister to human welfare. Future knowledge depends largely upon to-day's students and what I feel we need, above all things, is to arouse enthusiasm in our students, to strike their imagination, so that they shall go forward not only with accurate knowledge but with faith and vision, a faith that is deep and a vision that is wide and constructive. We must realise that, although as academic beings, it is worth while studying grass for the sake of studying grass yet, as human beings, it is perhaps an even nobler ideal to study grass for the sake of making two blades of grass grow where one only has grown before. And finally we must so teach and so wean our students that they shall have a more "gestalt" perspective and a more "holistic" philosophy, so that their ideology shall be one of relationships, in which the phenomena of health and disease are perceived, not as mere isolated things, but as events in the pattern of life and death. In such case I have little doubt that disease in plants is a matter for human control.

## REVIEWS

*Cotton and its Production.* By W. H. JOHNSON. Pp. xxvii + 536; 26 maps. London: Macmillan and Co., Ltd., 1926. 25s. net.

*Cotton.* By H. B. BROWN. Pp. 517; 139 figs. New York and London: McGraw-Hill Book Co., Ltd. 1927.

Since the publication by the United States Department of Agriculture in 1896 of its *Bulletin* No. 33, "The Cotton Plant, its history, botany, chemistry, culture, enemies and uses," no important treatise covering the same large field has appeared until the nearly simultaneous publication of these two manuals. Although both cover the whole field of cotton in all its aspects, the authors have approached the subject from different angles. In *Cotton and its Production* the outlook is mainly commercial, and the subject is treated geographically. Each of the cotton-growing areas of the world is considered in turn and their particular problems of cotton cultivation and production discussed. The purely botanical aspects of the genus *Gossypium* are dismissed in twelve pages, while cotton breeding is treated of in nine. One of the most interesting chapters is that on cotton growing in India. The area planted annually to cotton in India is only one-third less than the whole of the Cotton Belt of America, but owing to the inferior yields the crop is only one-third as large as the American crop. The average yield in India varies in different years from about 60 lbs. to 100 lbs. of lint per acre but has never exceeded 100 lbs.; the yield in America is at least twice this. While it is realised that this poor yield is due in part to unavoidable factors such as unfavourable climatic and soil conditions in many parts of the country, yet, as Mr Johnson points out, still more is it due to a lack of knowledge, and intelligence to apply knowledge, on the part of the peasant grower. A great deal has been done to improve this state of affairs by the Agricultural Departments, while the Co-operative Credit Societies have enabled the small-holder to obtain the capital he needs for his initial outlay without recourse to that prince of usurers, the native money-lender. How much has been done to improve the yield is seen when it is considered that for the five years before the War, 1909-14, the average yield per acre was 77 lbs. of lint, while for the five years 1920-25 the yield was 90 lbs. of lint per acre.

Of much interest too is the chapter on cotton growing in Australia. In 1920-21 Mr Johnson visited Australia on a special commission to enquire into the possibility of developing the cotton-growing industry, and the substance of his reports is contained in this chapter. A huge area in the continent is suitable for the growing of cotton but the extreme sparseness of the population makes its production on a large scale extremely difficult. The author points out that a farmer with a medium-sized family can do all the work on a cotton farm up to ten acres without outside assistance, the labour required for picking being the factor which fixes this limit. Great efforts are being made to improve the varieties grown and to see that the wholesale mixing of seed that has occurred in the past should cease. The export of Australian cotton has risen from 30 bales in 1919 to over 11,000 bales in 1925.

The chapter on the diseases of the cotton plant is not so good. The sketchiness of the treatment and the somewhat inaccurate statements made indicate only a slight acquaintance with the subject. A "boll rot," said to be due to *Bacillus gossypinus* Stedman, is described as being "commonly met with in the United States and the West Indies," whereas it was shown many years ago that Stedman's green fluorescent bacillus was not pathogenic. "Internal Boll Disease" caused by Nowell's Species A, B, C and D (*Nematospora*, *Eremothecium*, and *Spermophthora*) is dismissed in two sentences, despite its great economic importance in the West Indies. Insect pests are treated at greater length and in more detail.

Coming to Prof. Brown's volume we find the subject treated, as we should expect with a geneticist as author, far more from the botanical standpoint. The first seven chapters are concerned entirely with the botany of the cotton plant, its species, varieties, morphology, and reproduction. It is surprising that, considering the immense economic importance of the crop, so little research has been done on the physiology of the cotton plant. The monumental work of W. L. Balls in Egypt is almost the sole source of our knowledge. What data there are, however, are very ably presented by the author in this volume, and the physiological problems arising are interestingly discussed. Forty-three pages are then devoted to the genetics of the cotton plant and the known facts are excellently reviewed.

Soils, climate, and cultivation receive considerable attention.

Diseases are treated in rather more detail than in *Cotton and its Production*, and the information given is more in accordance with modern views. However, like many others the author accepts the attribution of Wilt Disease to *Neocosmospora vasinfecta* (Atk.) E. F. Smith, although the evidence on which this fungus was stated to be the ascigerous stage of *Fusarium vasinfectum* was more than inconclusive, and is discounted by most authorities on the subject. As the author states in the preface, the information in the book applies chiefly to the Upland cotton area of the American Cotton Belt, and this accounts for the extent to which "wilt disease" and "anthracnose," the most serious and widespread diseases in this area are discussed, while "Internal Boll Disease" does not even receive mention.

The commercial aspects are interestingly and clearly handled in both volumes, though perhaps more detailed and carefully illustrated accounts of the processes involved in spinning and weaving would enhance the value. Together the two books constitute a most comprehensive treatment of all aspects of cotton and its production.

The bibliographies are more complete in Prof. Brown's volume, the specific citations in Mr Johnson's work being confined to books only. The style of both volumes is excellent and maintains the standard set by these publishers. The maps in *Cotton and its Production* are particularly clear and valuable, although the book as a whole would have been improved by illustrations. *Cotton* is typical of the very high standard of the McGraw-Hill publications.

R. H. STOUGHTON.

*Economic Biology for Students of Social Science.* Part I. Harmful and Useful Animals. By PHILIPPA C. ESDAILE. Pp. xv + 175; 150 illustrations. University of London Press, Ltd., 1927. 7s. 6d. net.

The aim of this book is to present the elementary facts of economic biology from the standpoint of "Household and Social Science." It is an attempt to cover a rather ill-defined field which does not seem to have been catered for by any book of a similar character. Dr Esdaile's efforts aim at giving such information that is most generally useful concerning certain animals and plants which are, or may be, associated with man and the household. The present volume which forms Part I deals with harmful and useful animals, while Part II, it is mentioned in the Preface, constitutes a companion volume which treats of animal and plant products.

Such a work as Herrick's *Household Insects* covers only that branch of the subject as is implied by its title, while Dr Esdaile's book is of a much wider scope although less detailed in its method of treatment. The difficulty in writing a manual of this character lies in the choice of material, since the subject in its present aspect has not so far assumed very definite boundaries. Its authoress has taken a wide viewpoint and included all organisms from Protozoa to Arthropoda wherever they betray any relation to her subject. Vertebrates, it appears, do not come within the scope. Chapter I is concerned with the Protozoa and an account is given of the species of *Entamoeba* found in man, of the malaria Plasmodium and its relation to *Anopheles*, together with some brief remarks on the *Spirochaeta* of syphilis and the causation of that



disease. Chapter II deals with the Porifera and their general structure, together with a list of the economically important species and their special uses to man. Flat worms form the subject of Chapter III and a clear, but brief, account is given of the structure and life-histories of the liver fluke of the sheep and of tapeworms; while certain economically important round worms are dealt with in the next chapter. The Insecta form the subjects of Chapters V to XII and practically all the important species affecting man in person or his stored products, in so far as Britain is concerned, are mentioned. Spiders and economically important mites are dealt with in Chapter XIII and the concluding chapter is devoted to general hygienic measures. The book is also supplied with a glossary of the technical terms used, and an index.

We have little to offer by way of criticism, but in dealing with the Hymenoptera, reference might well have been made to ants which can be troublesome invaders of the household. Under the Diptera we miss any mention of the biting housefly *Stomoxys calcitrans* and in the chapter on Coleoptera *Attagenus pellio* would seem to require mention, since it is usually found in houses where its larva attacks furs, skins, etc. These few omissions scarcely detract from the value of the book and attention is directed towards them in the event of a second edition being called for later.

The general impression derived from reading this manual is clarity of expression, accuracy and strict elimination of wordiness. It is, in fact, rigidly confined to essentials, and we may say that the task is well done. Preventive or remedial measures are given wherever desirable and those advocated are up to date and eminently practical. The book is well adapted as an elementary manual and it should meet with a ready demand among the type of reader it is intended to cater for. It is clearly printed in good type and the illustrations are adequate.

A. D. IMMS.

*Genetics in Relation to Agriculture.* By E. B. BABCOCK and R. E. CLAUSEN.  
2nd ed. Pp. xiv + 673. McGraw-Hill Book Co. 1927. 25s. net.

Few aspects of science have shown so remarkable a development as modern genetics, which beginning almost *de novo* a quarter of a century ago, has acquired a mass of exact data and fundamental theory that will bear comparison with those of any other scientific discipline. This, in part, is due to the curiously post-dated birth of genetics and the correlated fact that a great amount of anatomical, cytological and other exact information and practical breeding experience were available which could be incorporated immediately as a broad and stable foundation for the new development. In the quite early years certain fruitful lines of investigation began to crystallise out: on the one hand, the study of the mechanism of Mendelian heredity and the relation of the gene to character effect and, on the other hand, the study of pure-line analysis and the more empirical splitting and synthesis of the phenotype. The genetic research of the last 25 years has been almost entirely an advance along these lines and the main congeries of obvious problems have been more or less explored, the greater number of geneticists being now engaged in filling in details or showing the application of already formulated theory to further examples. Broadly speaking, this, the first period of genetic development has come to an end and the science is in the throes of a re-birth. The atomistic ideology of genetics is in process of rapid change, a dynamic philosophy arising on the foundation of the static philosophy of the last quarter of a century. It is a transition that is clear not only in the more enclosed field of genetics but in the whole biological and psychological landscapes, mechanistic philosophies sublimating into hormic or dynamic philosophies. The atomistic ideology of genetics from 1900-25 might almost be regarded as a necessary pre-requisite for the emergent evolution of the science to a functional plane, a plane on which genetic phenomena are perceived as events rather than things. Of the one school of philosophy it might be said that the teachers are Johannsen and Morgan, of the other school, Bateson and Goldschmidt, with Baur as a bridge between the two schools and Lotay as *Advocatus Diaboli* in general. Such work as that of Goldschmidt (see for example his recently published *Physiologische Theorie der*

*Vererbung*, 1927) or particulate researches such as that of Plunkett, are not merely an additional new line of study but an entirely new viewpoint and a new plane of exploration. They are the foundation stones of a developmental genetics and a dynamic philosophy as against the older mechanistic genetics and static philosophy. There would seem little room for doubt that the science of physiological genetics opening before us will entirely re-orientate genetic theory and philosophy.

It is desirable that, at the close of a period, a book should be taken and the general position reviewed so that a vantage ground may be selected for further endeavour. This has been happening in genetics and the cognate disciplines. On the cytological side volumes have appeared by Cowdry (edit), Wilson and Sharp. In genetics itself are the monographic volumes by Fruwirth and the series edited by Baur and by Lohs and Kooimanns. Of single treatises covering the entire field or on one or another aspect of genetics are the volumes by Crew, Rice, Hayes and Garber, Shull, Sinnott and Dunn, Babcock and Clausen, Jones, Johannsen, Baur, Castle, Walter, Morgan and others. All these have been published during the last two or three years and to a greater or less extent review the position to date. Certain of these volumes are second editions and a comparison of two editions is always interesting for it enables one to measure the progress that has been made in a specified period. For example the time elapsing between the second and third editions of Wilson's *The Cell* corresponds with the life of modern genetics, 1900-25, and a comparison of the two editions gives an extraordinarily clear picture of the development of cytogenetics. If one compares the 1913 and 1926 editions of Johannsen's treatise one, similarly, obtains a view of progress in pure-line theory and practice. Perhaps the best picture of the advances in general genetics is obtainable from the several editions of Baur's volume, or from the three editions of Castle's treatise although this gives a more personal point of view.

An excellent idea of the general advance in genetic development made during the last decade is obtained by a comparison of the first edition of the work by Babcock and Clausen published in 1918 and the second edition which has just appeared. In 1918, for example, the fundamental importance of the chromosomes in genetic theory although strongly indicated might still be regarded as a moot point, whereas to-day, the value of cytogenetic research is no longer in question. The applied value of genetic research in animal and plant breeding was, in 1918, still largely a matter of hope and promise, whereas to-day much of that promise is not only well on its way to fulfilment but certain issues of primary horticultural or agricultural importance have been solved outright. It is interesting to contrast the development of genetics during this period and its outlook to-day with those of, for example plant pathology, one of the few aspects of biology which has shown an equally rapid growth. In plant pathology the development has been almost entirely empirical and the singular lack of fundamental research and theory has given rise to an unstable, because unbalanced, structure. In genetics, on the other hand, theory has advanced naturally and logically in the van of practical application, and perhaps nothing characterises the close of this youthful period of genetic development so much as the broad advance made in fundamental research and the stable balanced nature of the resultant growth.

The genetic progress during the period 1918-27 is well reflected in the difference between the two editions of Babcock and Clausen's volume. Both are divided into three parts—I. Fundamentals, II. Plant Breeding, and III. Animal Breeding, but whereas in the second edition it has been found possible to reduce Part III from 172 pages to 134 pages and Part II from 156 pages to 148 pages, the same process of condensation applied to Part I has necessitated an increase in number of pages from 286 to 330.

This fundamental advance is, of course, largely due to the work of Morgan and his collaborators and the first portion of the volume is principally based on the researches of the Columbia school to which it forms an excellent and simple introduction. The only broad criticism one would make of Part I is that it is perhaps too strongly coloured by the researches of the authors' compatriots—Morgan *et al.*, East, Emerson, Blakeslee, Jones, Castle and others and that not sufficient attention has been given to the often divergent views of such European investigators as Bateson,

Baur, Lotze, Clausen, Turesson, Nilsson and so forth. To take, for example, one issue only, that of sex, with its ramifying implications, the fundamental and revolutionary investigations of Goldschmidt, although referred to in the bibliography, are almost passed over in the text. In detail there are many points of interpretation in the authors' treatment of "Fundamentals" with which one finds it somewhat difficult to agree, but this is only to be expected in so new and rapidly developing a science as genetics. One rather more general issue which underlies the whole treatment of this first part may be noted. The authors have re-classified the phenomena of variation into I. "Developmental variation," II. "Mendelian variation," and III. "Mutation." The first category aligns with the older term "modification"; the second, in part with the older term "combination" but excludes the expression of aberrant or multiple chromosome re-arrangements which along with qualitative changes and all changes of unknown causation are relegated to the third category of "mutation." This last term thus connotes the expression of both qualitative and quantitative changes in germinal elements and is, as the authors state a "catchall" term. This new classification of variation is neither logical or particularly helpful to clear and precise thinking, and it is a pity that in a volume which is obviously destined to become a widely accepted and standard text some more logical categorisation such, for example, as that suggested by Baur in his original treatise ("Einführung in die experimentelle Vererbungslehre") or elaborated in detail more recently by Bridges, should not have been adopted.

Part II deals with plant breeding and is an excellent and concise introduction to an extraordinarily wide and diffuse subject. Even Fruchwart's five volumes only partially cover this field and in a book of the present size a preliminary reconnaissance only can be made. The note running through this portion—the urgent need for the most intimate cooperation between practical grower and genetical investigator—is one which cannot be too strongly emphasised.

Part III is concerned with the application of genetics to animal breeding and is a simple and straightforward introduction to what has already been accomplished and of the genetic facts and implications which underlie the occupation of the practical breeder. In a chapter on "acquired characters" much of the diffuse unsatisfactory work in this field is dealt with in an admirably critical way, but one would have liked to see some reference made to the genetic implications of such work, for example, that of Tornier. One of the most interesting chapters is that dealing with the promising and immensely important subject of animal hybridisation.

The "list of literature" runs to 34 pages as against 26 in the first edition but a number of these items are not referred to in the work, and one finds it a little difficult to understand the inclusion of many of these references, when other more important ones are omitted. No references are actually cited in the text and those at the ends of the chapters, a new feature of this edition, might in many cases have been omitted as they are merely to elementary text-books. The glossary and synoptic contents are absent from this second edition; the text-figures have been reduced from 239 to 203, many of which are new, and the coloured plates have been reduced from five to four of which three are new.

Taking it all in all, one may question, whether the new edition is quite as good a treatise on *Genetics in Relation to Agriculture* in 1927 as was the first edition on the same subject in 1918—certainly it is not as readable, but that is probably due to the difficulty of condensing so enormously expanded a mass of data into the same compass, 673 pages as against 675. In any case the book stands quite alone in its particular field and not only geneticists and agriculturists but all biologists owe a debt of gratitude to the authors for carrying out successfully so difficult and onerous a task.

WILLIAM B. BRIERLEY.





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